METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF TOLTERODINE TARTRATE IN TABLET DOSAGE FORM BY RP-HPLC

Dissertation

Submitted to

The Tamil Nadu Dr.M.G.R Medical University, Chennai.

In partial fulfilment for the award of the degree of

MASTER OF PHARMACY

In

PHARMACEUTICAL ANALYSIS

By

M.SHAJU

DEPARTMENT OF PHARMACEUTICAL ANALYSIS

ULTRA COLLEGE OF PHARMACY

4/235,COLLEGE ROAD,THASILDAR NAGAR,

MADURAI-625020.

APRIL 2013

DECLARATION

I hereby declare that this thesis work entitled " **METHOD DEVELOPMENT AND METHOD VALIDATION FOR THE ESTIMATION OF TOLTERODINE TARTRATE IN TABLET DOSAGE FORM BY RP-HPLC "** submitted to The Tamil Nadu Dr.M.G.R Medical University, Chennai was carried out by me in the Department of Pharmaceutical Analysis, Ultra College of Pharmacy, Madurai under the valuable and efficient guidance of **Mr.V.SIVANAND**, **M.Pharm.,** Professor , Department of Pharmaceutical Analysis , Ultra College of Pharmacy, Madurai during the academic year May2012- March 2013. I also declare that the matter embodied in it is a genuine work and the same has not formed the basis for the award of any degree, diploma, associateship, fellowship of any other university or institution.

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Apart from my efforts, the success of this project depends largely on the encouragement and guidance of many others. I take the privilege and pleasure to express my gratitude to the people who have been instrumental in the successful completion of this project.

I express my extreme sense of gratitude and profound thanks to my guide**, Mr.V.SIVANAND, M.Pharm., Ph.D., Asst Professor,** Department of Pharmaceutical Analysis, for his precious guidance, encouragement, abundant help and timely suggestions which proved for the success of this work.

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CONTENTS

INTRODUCTION[1,2,3,4]

Living things are those, which make the entire world productive and interesting. The absence of the living things proves the world futile. Among the living things such as microorganism, plants, animals and human, it is the human being, which predominates, and there by leads and of course rules over the other.

As the days moves on rapidly it is a noted fact that the life style, culture and the environment of the mankind has changed tremendously. In this rapidly changing world the emergence of various life threatening illness, diseases and other serious syndromes have become vary common and also a part of life. In order to eradicate the emergence of the new diseases and also to get rid out of various existing diseases. The inventions of newer agents or newer molecules become the concern.

In the act of invention of newer agents and molecules there are various aspects to the considered such as easy availability, affordability, low manufacturing cost, high efficacy and with minimal side effects.

Considering the various above said factors the medicinal chemists throughout the world are seriously as well as sincerely involved in the newer drug synthesis and it determination to enable as well as to enhance the well being of the mankind.

The hundreds or thousands of new organic chemicals are prepared annually throughout the world and many of them entered into pharmacological screens to determine whether they have useful biological activity. This process of random screening has been considered inefficient but it has resulted in the identification of new drugs and it have been determined quantitatively.

ANALYTICAL CHEMISTRY[1,4,7]

Analytical chemistry is a scientific discipline used to study the chemical composition, structure and behaviour of matter. The purpose of this is to gather and interpret chemical information that will be of value to society in a wide range of contexts.

Any chemical analysis can be broken down into a number of stages that include a consideration of the purpose of the analysis, the quality of the results required and the individual steps in the overall analytical procedure. Analytical procedure summarized by the following steps.

1. Definition of the problem.
2. Choice of technique and method.
3. Sampling
4. Sample pretreatment or conditioning
5. Qualitative analysis
6. Quantitative analysis
7. Preparation of report or certificate of analysis
8. Review of the original problem.

QUANTITATIVE ANALYSIS:

It is the determination of the absolute or relative amounts of elements, species or compounds present in sample.

QUALITATIVE ANALYSIS:

It is the identification of elements, species and/or compounds present in a sample.

ANALYTICAL TECHNIQUE:

There are numerous chemical or physico-chemical processes that can be used to provide analytical information. The processes are related to a wide range of atomic and molecular properties and phenomena that enable elements and compounds to be detected and/or quantitatively measured under controlled conditions. Instrumental techniques are used for much analysis and constitute the discipline of instrumental analysis. Atomic or molecular spectrometry and chromatography, which together comprise the largest and most widely used groups of techniques, can be further subdivided according to their physico chemical basis.

ANALYTICAL METHODS

Analytical methods are a detailed set of instructions for a particular analysis using a specified technique. Many standard analytical methods have been published as papers in analytical journals and other scientific literature, and in textbook form often, laboratory will develop their own "in-house methods" or adapt existing ones for specific purposes.

Method development forms a significant part of the work of most analytical laboratories and method validation and periodic revalidation is a necessity. Analytical methods must be shown to give reliable data, free from bias and suitable for the intended use. Most methods are multi step procedures, and the process of validation generally involves a stepwise approach in which optimized experimental parameters are tested for robustness that is sensitivity to variations in the conditions and sources of errors investigated.

The instrument is only one component of the total analysis. Often it is necessary to use several instrumental techniques to obtain the information required to solve an analytical

problem. Analytical chemists to save time, to avoid chemical separation or to obtain increased accuracy, may use instrumental method. The time saving feature can be realized in routine analysis, or where a considerable number of determinations are to be made. Most instrumental techniques fit into one of the three principal areas: Spectroscopy, Electrochemistry and Chromatography.

PRINCIPLE TYPES OF CHEMICAL INSTRUMENTATION:

A. Spectrometric techniques:

- Ultra violet and Visible Spectrophotometry
- Fluorescence and Phosphorescence Spectrophotometry
- Atomic Spectrometry (Emission and Absorption)
- Infrared Spectrophotometry
- Raman Spectroscopy
- X-ray Spectroscopy
- Radiochemical techniques including activation analysis
- Nuclear Magnetic Resonance Spectroscopy
- Electron Spin Resonance Spectroscopy

B. Chromatographic techniques

- Gas Chromatography
- High Performance Liquid Chromatography
- Thin layer Chromatography

C. Electrochemical techniques

- Potentiometry
- Voltametry
- Stripping Techniques
- Amperometric Techniques.
- Colorimetry
- Electrogravimetry
- Conductance Techniques

D. Miscellaneous Techniques

- Thermal Analysis
- Mass Spectrometry
- Kinetic Techniques

E. Hyphenated Techniques:

- GC-MS (Gas Chromatography - Mass Spectrometry)

- ICP-MS (Inductivity Coupled Plasma Mass Spectrometry)
- GC-IR (Gas Chromatography - Infrared Spectroscopy)
- MS-MS (Mass Spectrometry - Mass Spectrometry)

ANALYTICAL DEVELOPMENT:[4,7,8,9,10]

Devising accurate assay procedures for each ingredient of complex dosage formulations containing several therapeutically and chemically compatible drugs with very similar chemical nature is a monumental undertaking. Separation, identification and estimation of each ingredient in such complex formulations is a challenging task.

Not only are the multiple active constituents present but also they are usually there in widely divergent concentration depending upon their relative potency and therapeutic need of the patient.

The presence of excipients, additives and decomposition products further complicates the analysis. Therefore analytical development is done for new drugs where no compendial methods are available. Or, alternate method development for existing (Non Pharmacopoeial) products to reduce cost and time of analysis.

Method development is done for:

1. New products.
2. Existing products

Methods are developed for new products when no official methods are available. Alternate methods for existing (Non- Pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness. Trial runs are conducted, method is optimized and validated.

When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available.

Selection of analytical method:

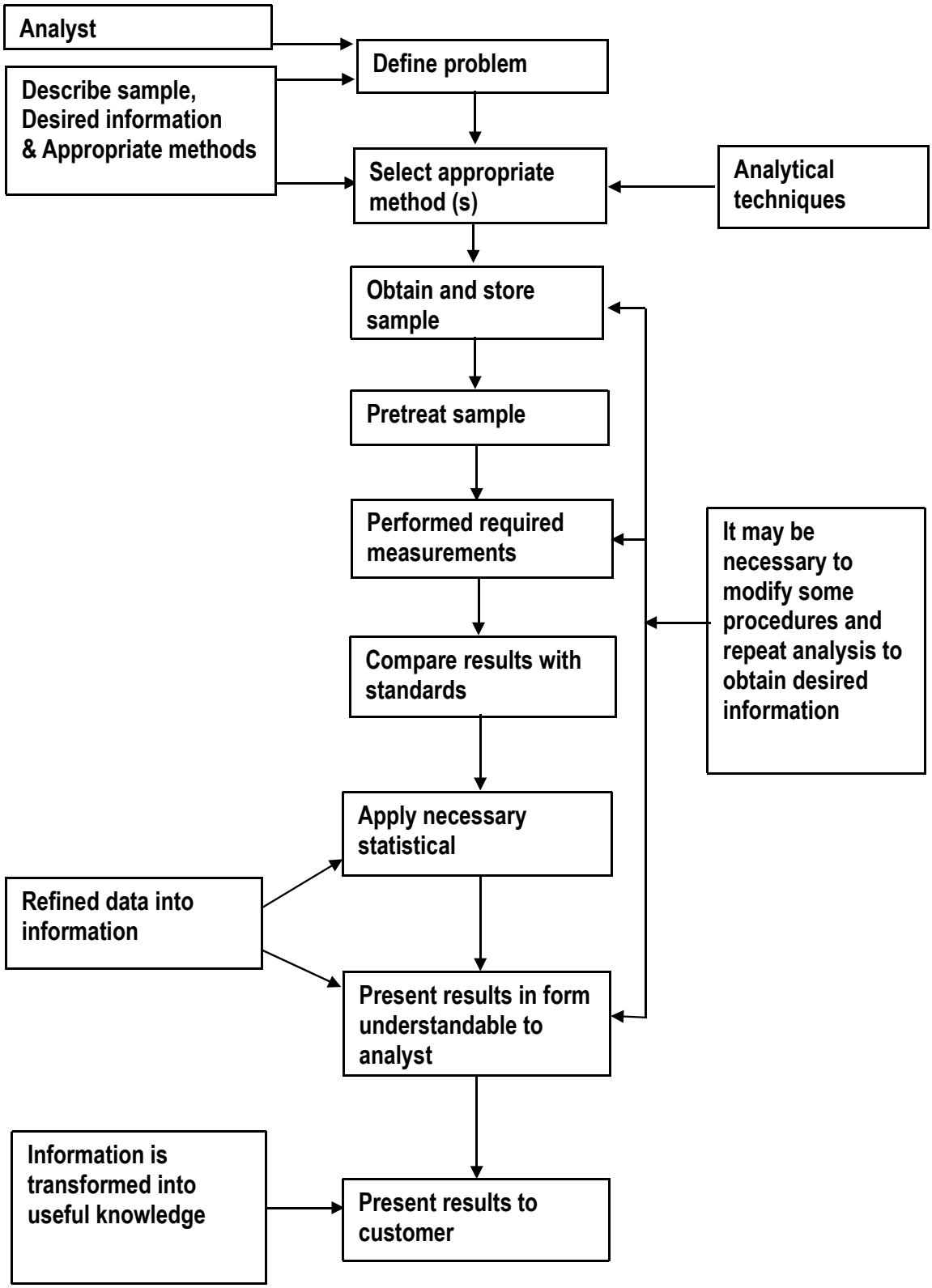
First stage in the selection or development of method is to establish what is being measured and how accurately it should be measured.

The following analytical techniques are usually employed for estimations different components in formulations:

1. Titrimetric and Gravimetric.
2. Ultraviolet and visible Spectrophotometry.
3. Thin layer chromatography.
4. High Performance Liquid Chromatography (HPLC)
5. Gas Chromatography (GC)

6. Atomic absorption Spectrometry (AAS)
7. Infrared absorption Spectrophotometry.

MAJOR STEPS IN SOLVING AN ANALYTICAL PROBLEM



METHOD DEVELOPMENT:

For the development of an analytical method various steps are followed which are considered essential for establishing the required documental evidence to prove that the said method is ideal and reproducible. The various steps followed in developing an analytical method is shown in following table:

STEPS OF METHOD DEVELOPMENT:

Documentation starts at the very beginning of the development process, a system for full documentation of the development studies must be established. All data relating to these studies must be established. All data relating to these studies must be recorded in laboratory notebook or an electronic database.

1. **Analyte standard characterization:**
   - All known information about the analyte and its structure is collected i.e. physical and chemical properties, toxicity, purity, hygroscopic nature, solubility and stability.
   - The standard analyte (100% purity) is obtained. Necessary arrangement is made for the proper storage (refrigerator, desiccators, freezer)
   - When multiple components are to be analyzed in the sample matrix, the number of components is noted, data is assembled and the availability of standards for each one is determined.
   - Special attention has to be paid when samples are limited (small volume or mass) or an analyte is present at trace levels, it is noted.
   - Only those methods (MS, GC, HPLC etc.) that are compatible with sample stability are considered.
2. **Method Requirements:**

   The goals or requirements of the analytical method that need to be developed are considered and the analytical figures of merit are defined. The required detection limits, selectively, linearity, range, accuracy and precision are defined.
3. **Literature search and prior methodology:**

   The literature for all types of information related to the analyte is surveyed. For synthesis, physical and chemical properties, solubility and relevant analytical methods. Books, periodicals, chemical manufactures and regulatory agency compendia such as USP/NF, AOAC and ASTM publications are reviewed. Chemical Abstracts Service (CAS) automated computerized literature searches are convenient.

4. **Choosing a method:**
    - Adaptation is more efficient than "reinventing the wheel". If any of the reported methods from the literature are adaptable to the current laboratory setting and future needs, it is determined.
    - Using the information in the literatures and prints, methodology is adapted. The methods are modified wherever necessary. Sometimes it is necessary to acquire additional instrumentation to reproduce, modify, improve or validate existing methods for in-house analytes and samples.
    - If there are no prior methods for the analyte in the literature, from analogy, the compounds that are similar in structure and chemical properties are investigated and are worked out. There is usually one compound for which analytical method already exist that is similar to the analyte of interest.
5. **Instrumental setup and initial studies:**
    - The required instrumentation is set up. Installation, operational and performance qualification of instrumentation using laboratory standard operating procedures (SOP's) are verified.
    - Always new consumables (e.g. Solvents, filters and gases) are used, for example, method development is never started, on a HPLC column that has been used earlier.
    - The analyte standard in a suitable injection / introduction solution and in known concentrations and solvents are prepared. It is important to start with an authentic, known standard rather than with a complex sample matrix. If the sample is extremely close to the standard (e.g. bulk drug), then it is possible to start work with the actual sample.
    - The analysis is done using analytical conditions described in the existing literature.
    - Feasibility of method with regard to the analytical figures of merit obtained is evaluated.
6. **Optimization:**

    During optimization one parameter is changed at a time, and set of conditions are isolated, rather than using a trial and error approach. Work has been done form an

organized methodical plan, and every step is documented (in a lab notebook) in case of dead ends.

7. **Documentation of analytical figures of merit:**

   The originally determined analytical figures of merit Limit of Quantitation (LOQ), Limit of Detection (LOD), linearity, time per analysis, cost, sample preparation etc are documented.

8. **Evaluation of method development with actual samples:**

   The sample solution should lead to unequivocal, absolute identification of the analyte peak of interest from all other matrix components.

9. **Determination of percent recovery of actual sample and demonstration of quantitative sample analysis:**

   Percent recovery of spiked, authentic standard analyte into a sample matrix that is shown to contain no analyte is determined. Reproducibility of recovery (average +/- standard deviation) from sample to sample and whether recovery has been optimized has been shown. It is not necessary to obtain 100% recovery as long as the results are reproducible and known with a high degree of certainty.

The validity of an analytical method can be verified only by laboratory studies. Therefore documentation of the successful completion of such studies is a basic requirement for determining whether a method is suitable for its intended applications.

Brief description of the various analytical methods:

B. **SPECTROSCOPY:**

In Spectrometric methods, the sample solution absorbs Electromagnetic Radiation from an appropriate source, and the amount absorbed is related to the concentration of the analyte in solution.

UV AND VISIBLE SPECTROSCOPY:

Ultraviolet and visible radiation is involved with electronic excitation. Spectroscopically, UV and visible radiation operates with wavelength between 20-800nm. The UV and visible spectroscopy is based on the principle of beer-lamberts law relating to intensity of light with thickness and concentration of medium. The UV and visible spectroscopy is concerned with the quantitative analysis and structural elucidation. The instrumentation of UV and visible spectrophotometer consists of radiation source; monochromators, sample cells, detectors, recording systems, powering supply. They are Double beam and Single beam spectrophotometers. Ultraviolet and visible spectrometric method is suitable when no interference is observed in the mixture.

CHROMATOGRAPHY

It is the process of separating the components of mixtures (solutes) that are distributed between a stationary phase and a flowing mobile phase according to the rate at which they are transported through the stationary phase.

Chromatographic technique can be classified according to whether the separation takes place on a planar surface or in a column. They can be further subdivided into gas and liquid chromatography, and by physical form, solid or liquid of the stationary phase and the nature of the interactions of solutes with it known as sorption mechanism.

HPLC -- HIGH PRESSURE (PERFORMANCE) LIQUID CHROMATOGRAPHY

High Performance Liquid Chromatography (HPLC) is a chemistry based tool for quantifying and analyzing mixtures of chemical compounds. It's used to find the amount of a chemical compound within a mixture of other chemicals. High Performance Liquid Chromatography (HPLC) is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial, etc. In a liquid chromatographic process a liquid permeates through a porous solid stationary phase and elutes the solutes into a flow-through detector. The stationary phase is usually in the form of small-diameter (5-10 mm) uniform particles, packed into a cylindrical column. The typical column is constructed from a rigid material (such as stainless steel or plastic) and is generally 5-30 cm long and the internal diameter is in the range of 1-9 mm

1. Increase resolution of liquid chromatography by...

    - Use very small particles to get the large possible surface area → 3 - 20 μ diameter
    - High pressure (up to 400 atmospheres) needed to get acceptable flow rates → requires very strong particles to resist bed compression and crushing of particles.
    - Tens of thousands of "plates" per meter of column length provides excellent resolution

1 Used in most of the normal modes discussed above although reversed phase is probably the most common type of HPLC

2 Advantages: very high resolution (replaces Paper chromatography / Electrophoresis in most applications) and short run times.

REVERSED PHASE CHROMATOGRAPHY

1. Stationary Phase -- Apolar (hydrophobic) → reversed with respect to cellulose chromatography

    - Hydrocarbon chains -- bound to an inert matrix; hydrophobicity varied by changing the hydrocarbon chain length
    - Aromatic groups

1 Mobile Phase -- depends upon hydrophobicity of stationary phase. Commonly use a more polar organic solvent: Acetonitrile, DMSO, EtOH, Ethylene glycol, Propanol, or mixtures of these with H_2O. Also may use gradients.

CHROMATOGRAPHY SCALE:

- Analytical - Just Data [High Sensitivity]
- Semi-Preparative - Data and a small amount of purified analyte (gram)
- Preparative - Larger quantities of purified analytes (Kilograms) [High Capacity]

TYPES OF CHROMATOGRAPHY

There are three main types of chromatography, categorized by the mobile phase type:

- Gas (GC)
- Liquid (LC)
- Supercritical fluid (SFC)

·**MOBILE PHASE**:

- Type of modifier (MeoH, Acetonitrile)
- Solvent Strength
- PH
- Type of buffer(Phosphate, acetate)
- Ionic strength (salts, Buffer concentration)
- Ion-Pairing Reagents (alkylamines, -sulfonates)

CHIRAL STATIONARY PHASES

- Ligand exchange
- p-Donor p-acceptor (Pirkle)
- Chiral Host-guest (cyclodextrin)
- Immobilized proteins
- Immobilized polysaccharides

This is a highly specific mode of chromatography in which molecular recognition process takes place between the agents bonded to the stationary phase and the solutes. The principle of lock-key recognition similar to enzymes takes place. In size exclusion chromatography molecules are separated based on their molecular size in a sieving effect. The bigger molecules (higher molecular weight) elute earlier.

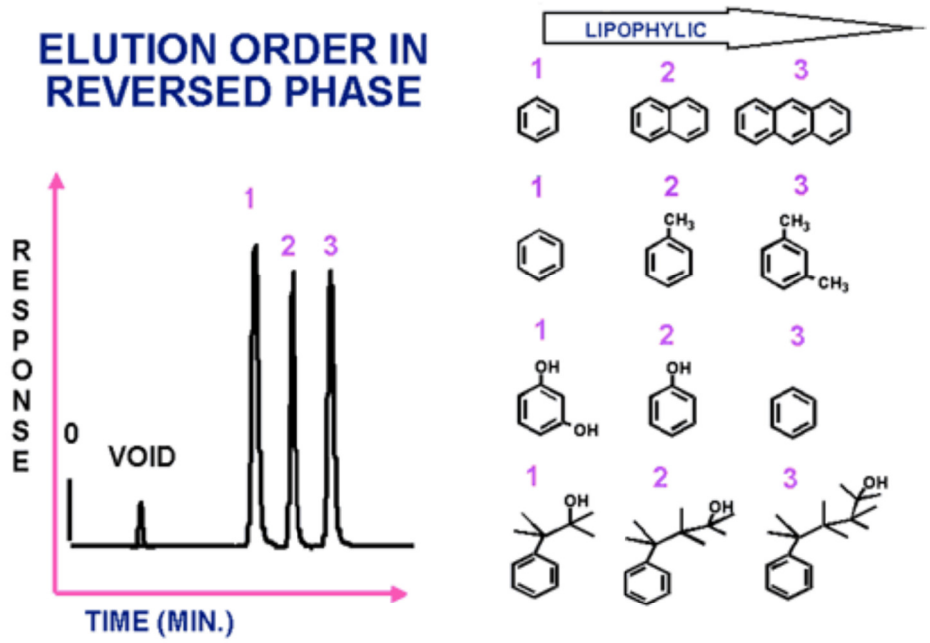
The system is calibrated using standards of known molecular weights and the unknown's molecular size distribution is determined from the calibration curve. Retention times are related to the log MW (logarithm of molecular weight). There are many variables to consider when selecting the mode of chromatography to work with. Since most of the applications use Reversed Phase, all these variables are taken into consideration during method development for this mode of HPLC.

HPLC COLUMNS:

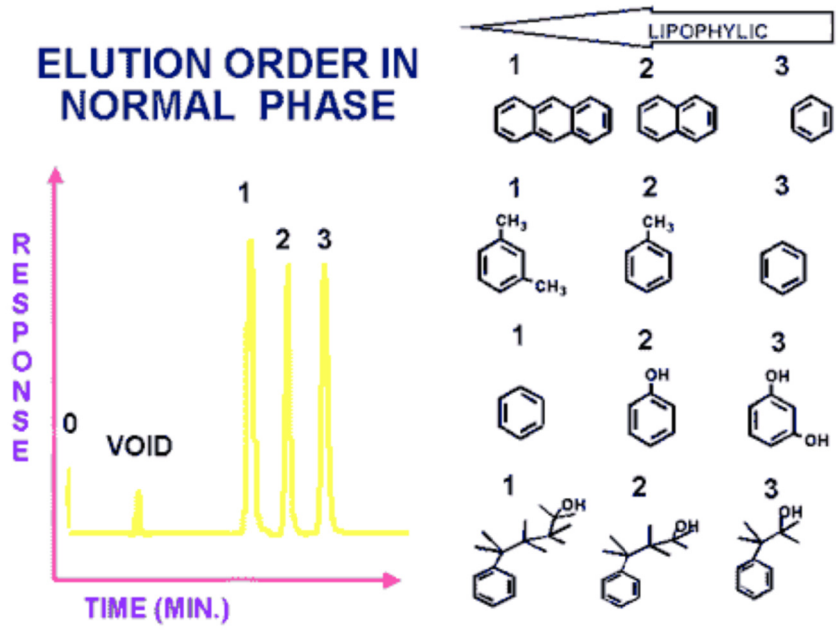
1. The most efficient columns produce the sharpest peaks, which gives better separation by minimizing band spreading

2. A tight narrow sample “band” is produced when a column’s stationary phase is uniformly packed (mechanical)

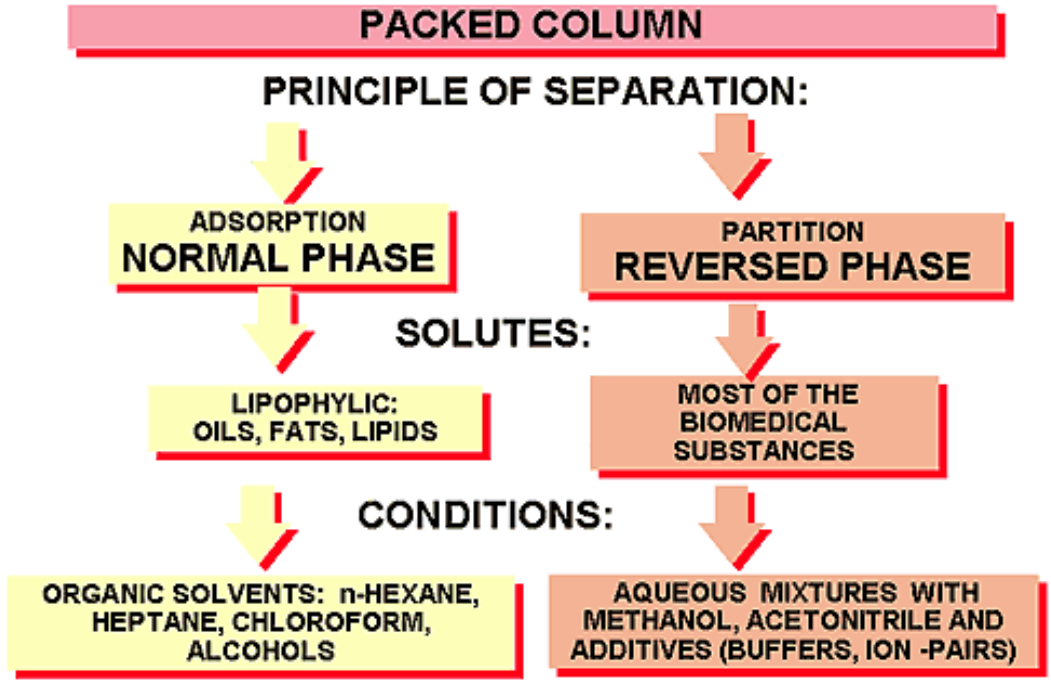
3.The packing material adds “chemical” band spreading (tailing, pore depth etc.).



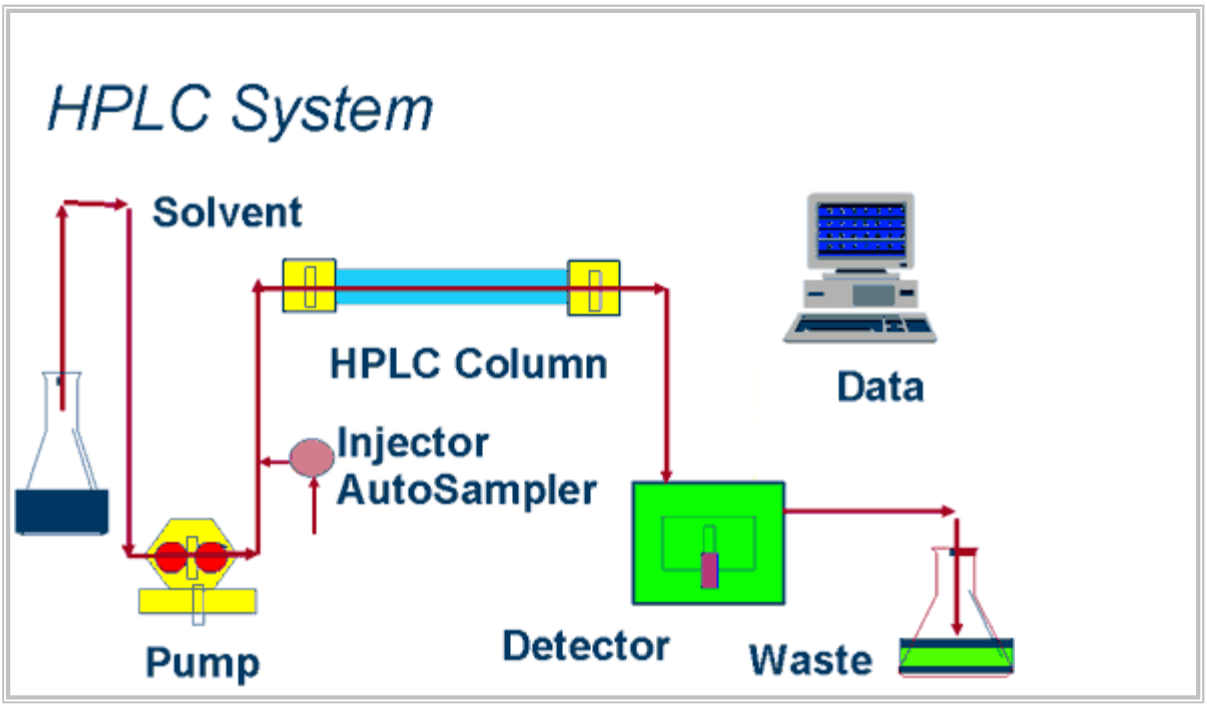
In Reversed Phase separations organic molecules are separated based on their degree of hydrophobicity. There is a correlation between the degree of lipophylicity and retention in the column. This is the list of mobile phase parameters effecting retention and separation in Reversed Phase.



Elution order in Normal Phase HPLC shows that the polar solutes elute later than non-polar lypophilic ones.



Separation can be achieved by controlling and manipulating these interactions, which effect the relative retention times of the various sample components.



DATA SYSTEM - A means of controlling the system components and storing, processing and displaying data

PUMPS:

A high pressure pump is required to force the mobile phase through the column at typical flow rates of 0.1-2 ml/min. The sample to be separated is introduced into the mobile phase by injection device, manual or automatic, prior to the column.

DETECTORS:

Instrument in the chromatographic system which senses the presence of a compound passing through, and provides an electronic signal to a recorder or computer data station. Output is usually peaks, that is, the chromatogram.

TYPES OF DETECTORS:

- UV – Ultraviolet light
    - Lamp
    - Grating/Lens - Wave length
    - Flow Cell
    - Photo Diode - Differential Light Output
- RI – Refractive Index
    - Universal analyte detector
    - Solvent must remain the same throughout separation
    - VERY temperature sensitive
    - Sometimes difficult to stabilize baseline
- FD – Fluorescence
    - Excitation wavelength generates fluorescence emission at a higher wavelength
    - Analytes must have fluorophore group
        - Can react analyte with fluorophore reagent
    - Very sensitive and selective
    - More difficult methods transfer
    - Results very dependent upon separation conditions
- MS – Mass Spectrometer
    - Mass to charge ratio (m/z)
    - Allows specific compound ID
    - Several types of ionization techniques

- Electrospray, Atmospheric pressure chemical ionization, electron impact

The detector usually contains low volume cell through which the mobile phase passes carrying the sample components. Choosing a Detector.

Criteria	RI	UV/VIS	Fluor.	MS
Response	**Universal**	**Selective**	**Selective**	**Selective**
Sensitivity	**4 microgram**	**5 nanogram**	**3 picogram**	**1 picogram**
Linear Range	**10**	**10**	**10**	**10**
Flow Sensitive	**Yes**	**No**	**No**	**Yes**
Temp. Sensitive	**Yes**	**No**	**No**	**No**

Seven Basic Considerations in Choosing HPLC Operating Parameters:

1. Solubility - Hexane, Chloroform, Methanol, Water (buffer pH), other
2. Molecular Weight - Would GPC be useful in either the analysis or sample prep?
3. Functional Groups - Any ionizable groups? Acidic, Basic, or Neutral?
4. Sample Matrix - What amounts are expected in matrix for either analytical or preparative isolation?
5. Levels in Matrix - What amounts are expected in matrix for either analytical or preparative isolation?
6. Detectability - Any chromophores or fluorophores? Consider Redox or derivatization. Together with point #5, an appropriate detector is chosen.
7. How Do Species Differ - An important clue to manipulate selectivity in the separation, especially if compounds are similar in their structure.

VALIDATION:[15,16]

Validation is an approach to form a basis for written procedures for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport or are represented possess.

Validation is defined as follows by different agencies:

FOOD AND DRUG ADMINSTRATION (FDA):

Establishing documentation evidence, which provides a high degree of assurance that specific process, will consistently produce a product meeting its predetermined specification and quality attributes.

WORLD HEALTH ORGANISATION (WHO):

Action of providing that any procedure, process, equipment, material, activity or system actually leads to the expected results.

EUROPEAN COMMITTEE (EC):

Action of providing in accordance with the principles of good manufacturing practice, that any procedure, processes, equipment material, activity or system actually lead to the expected results.

In brief validation is a key process for effective Quality Assurance. "Validation is establishing documental evidence which provides a high degree of assurance that a specific process or equipment will consistently produce a product meeting its predetermined specification and quality attributes".

OBJECTIVE OF VALIDATION:

The primary objective of validation is to form a basis for written procedures for production and process control which are designed to assure that the drug products have the identity, strength, quality and purity they purport or are represented to possess.

- Quality, safety and efficacy must be designed and built into the product.
- Each step of the manufacturing process must be controlled to maximize the probability that the finished product meets all quality and design specifications.

BENEFIT:

- QUALITY: Customer-patient satisfaction. It has been built into the product.
- UNDERSTANDING EQUIPMENT, SYSTEMS, PROCESSES: Process improvement, technology transfer, related product validation, rapid failure investigations, increased employee awareness.
- COST REDUCTION:
  - Increased efficiency, shortening lead-time resulting in lower inventories.
  - Fewer rejects and reworks.

- Longer equipment life by operating the equipment as per manufacturer's specifications and the establishing of cost effective preventive maintenacne schedules.
- Possible reduced testing of raw materials bulk formulations and finished products

❖ REGULATORY:

- Successful inspections
- Approved products
- Ability to export.

PHASES OF VALIDATION:

- Design Qualification (DQ): Documented verification of the design of equipment and manufacturing facilities.
- Installation Qualification (IQ): Documented verification of equipment or system design and adherence to manufacturer's recommendations.
- Operational Qualification (PQ): Documented verification that equipment or system performance in the target operating range.
- Process performance Qualification (PQ): Documented verification that equipment or systems operate as expected under routine production conditions. The operation is reproducible, reliable and in a state of control.
- Process / Product Validation: Validation is establishing documented evidence, which provides a high degree of assurance that a specific process will consistently produce a product meeting its pre-determined specifications and quality attributes.

TYPES OF VALIDATION:

➢ **PROSPECTIVE VALIDATION**: This is performed for all new equipment, products and processes. It is a proactive approach of documenting the design, specifications and performance before the system is operational. This is the most defendable type of validation.

➢ **CONCURRENT VALIDATION:** This is performed in two instances i.e. for existing equipment; verification of proper installation along with specific operational tests is done. In case of an existing, infrequently made product, data is gathered from at least three successful trials.

➢ **RETROSPECTIVE VALIDATION**: This is establishing documented evidence that the process is performed satisfactorily and consistently over

time, based on review and analysis of historical data. The source of such data is production and QA/QC records. The issues to be addressed here are changes to equipment, process, specifications and other relevant changes in the past.

DATA ELEMENTS REQUIRED FOR ASSAY VALIDATION:

Most common categories of assays for which validation data should be required. These categories are as follows.

CATEGORY I: - Analytical methods for quantitation of major components of bulk drug substances or active ingredients (including preservatives) in finished pharmaceutical products.

CATEGORY II: - Analytical methods for determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products. These methods include quantitative assays and limit tests.

CATEGORY III: - Analytical methods for determination of performance characteristics (e.g. dissolution, drug release)

CATEGORY IV: - Identification test.

For each assay category, different analytical information is needed. Data elements that is normally required for each of the categories of assays.

DATA ELEMENTS REQUIRED FOR VARIOUS CATEGORIES OF ASSAYS					
Analytical performance characteristics	Assay category I	Assay category II		Assay category III	Assay category IV
		Quantitative	Limit test		
Accuracy	Yes	Yes	¤	¤	No
Precision	Yes	Yes	No	Yes	No
Specificity	Yes	Yes	Yes	¤	Yes
Detection limit	No	No	Yes	¤	No
Quanititation limit	No	No	No	¤	No
Linearity	Yes	Yes	No	¤	No
Range	Yes	Yes	¤	¤	No

¤ → may be required, depending on the nature of the specific test

ANALYTICAL METHOD VALIDATION:

Analytical monitoring of a pharmaceutical product or of specific ingredients within the product is necessary to ensure its safety efficacy throughout all phases of its shelf life. Such monitoring is in accordance with the specifications elaborated during product development. Analytical validation is the cornerstone of process validation without a proven measurement system it is impossible to confirm whether the manufacturing process bas done what it purports to do. All new methods developed are validated.

Steps followed for validation procedures:

1. Proposed protocols or parameters for validations are established
2. Experimental studies are conducted.
3. Analytical results are evaluated.
4. Statistical evaluation is carried out.
5. Report is prepared documenting all the results.

OBJECTIVE:

The objective of validation of an analytical procedure is to demonstrate that is suitable for its intended purpose. Validation of analytical methods is the process by which it is established laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical applications. According to ICH, typical analytical performance characteristics that should be considered in the validation of the types of methods are

1. Accuracy
2. Precision
3. Specificity
4. Detection limit
5. Quantitation limit
6. Linearity
7. Range

The ICH documents give guidance on the necessity for revalidation in the following circumstances

1. Changes in the synthesis of the drug substances.
2. Changes in the composition of the drug product, and
3. Changes in the analytical procedures.

ANALTICAL PERFORMANCE CHARACTERISTICS:

ACCURACY:

Accuracy is the closeness of test results obtained by that method to the true value. The accuracy of an analytical method should be established across its range.

Determination of accuracy:

In case of assay of a drug substance accuracy may be determined by application of the analytical method to an analyte of known purity (e.g. reference standard) or by comparison of the results of the method with those of a second well characterized method, the accuracy of which has been stated or defined. Accuracy is calculated as the percentage of recovery by the assay of the known added amount of analyte in the sample, or as the difference between the mean and the accepted true value, together with confidence intervals. The ICH documents recommended that accuracy should be assessed using a minimum of nine determinations over a minimum of three concentration levels, covering the specified range (i.e., three concentrations and three replicates of each concentration)

PRECISION:

Precision is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of a homogenous sample. Precision of an analytical method is usually expressed as the standard deviation or relative standard deviation (coefficient of variation) of a series of measurement. Precision may be measure of either the degree of reproducibility or repeatability of the analytical method under normal operating conditions.

Determination of precision:

Precision of an analytical method is determined by assaying a sufficient number of aliquots of a homogenous sample to be able to calculate statistically valid estimates of standard deviation or relative standard deviation (coefficient of variation). The ICH documents recommend that repeatability should be assessed using a minimum of nine determinations covering the specified range for the procedure.

SPECIFICITY:

ICH documents defines specificity as the ability to assess unequivocally the analyte in the presence of compounds that may be expected to present, such as impurities, degradation products and matrix components.

IDENTIFICATION TESTS:

It ensure the identify of an analyte.

PURITY TEST: -

It ensures that all the analytical procedures performed allow an accurate statement of the content of impurities of an analyte. (e.g. related substances test, heavy metals limit, Organic volatile impurity test).

ASSAY:

It provides an exact result, which allows as accurate statement on the content or potency of the analyte in a sample.

SPECIFICITY:

ICH documents state that when chromatographic procedures are used, representative chromatograms should be presented to demonstrate the degree of selectivity and peaks should be appropriately labeled. Peak purity tests (e.g. Using diode array or mass spectrometry) may be useful to show that the analyte chromatographic peak is not attributable to more than one component.

DETECTION LIMIT:-

Characteristic of limit tests: Lowest amount of analyte in a sample that can be detected, but not necessarily quantities as an exact value, under the stated experimental conditions. The detection limit is usually expressed as the concentration of analyte (e.g. percentage parts per million) in the sample.

Determination of detection limit:

For instrumental and non-instrumental methods detection limit is generally determined by the analysis of samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be reliably detected.

In case of instrumental analytical procedures that exhibit background noise, the ICH documents describe a common approach which is to compare measured signals from samples with known low concentrations of analyte with those of blank samples. The minimum concentration at which the analyte can be reliably detected is established. Typically acceptable signal-to-noise ratios are 2:1 or 3:1. Whatever method is used, the detection limit should be subsequently validated by the analysis of a suitable number of samples known to be near, or prepared at the detection limit.

QUANTITATION LIMIT:

It is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. Quantitation limit is expressed as the concentration of analyte (e.g. Percentage parts per billion) in the sample.

Determination of Quantitation limit:

For instrumental and non-instrumental methods, the quantitation limit is generally determined by the analysis of samples with known concentration of analyte and by establishing the minimum level at which the analyte can be determined with acceptable accuracy and precision. In case of instrumental analytical methods that exhibit background noise, the ICH documents describe a common approach, which is to compare measured signals from samples with known low concentration of analyte with those of blank samples. A typically acceptable signal-to noise ratio is 10:1. Whatever method is used, the quantitation limit should be subsequently validated by the analysis of a suitable member of samples known to be near, or prepared at, the quantitation limit.

LINEARITY AND RANGE:

Linearity of an analytical method is its ability to elicit test results that are directly or by a well-defined mathematical transformation, proportional to the concentration of analyte in samples within a given range. The range of an analytical method is the interval between the upper and lower levels of analyte (including these levels).

That has been demonstrated to be determined with a suitable level of precision, accuracy and linearity using the method as written. Range is normally expressed in the same units as test results (e.g. found parts per million) obtained by the analytical method.

Determination of linearity and range:

Linearity should be established across the range of the analytical procedure. Initially by visual examination of a plot of signals as a function of analyte concentration of content. If linear relationship appears, test results are established by appropriate statistical methods (e.g. by calculation of a regression line by the method of least squares)

The range of the method is validated by verifying that the analytical method provides acceptable precision, accuracy, and linearity when applied to sample containing analyte at the extremes of the range as well as within the range. ICH recommends that for the establishment of linearity, a minimum of five concentrations normally be used.

RUGGEDNESS:

Degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments etc. Normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in condition normally expected from laboratory to laboratory and from analyst to analyst.

Determination of ruggedness:

By analysis of aliquots from homogenous lots in different laboratories, by different analysis, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. Degree of reproducibility of test results is then determined as a function of the assay variables.

SYSTEM SUITABILITY:-

According to USP system suitability are an integral part of chromatographic methods. These tests verify that the resolution and reproducibility of the system are adequate for the analysis to be performed. One consequence of the evaluation of robustness and ruggedness should be that a series of system suitability parameters is established to ensure that the validity of the analytical method is maintained whenever used.

System suitability tests are based on the concept that the equipment, electronics, analytical operations and samples constitute an integral system that can be evaluated as a whole.

ROBUSTNESS:

Robustness of an analytical method is measure of its capacity to remain unaffectedly small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

The parameters, which are recommended by International Committee of Harmonization to be validated for different types of assays, are shown in following table

INTERNATIONAL COMMITTEE ON HARMONIZATION (ICH) RECOMMENDATION	
ASSAY TYPE	**VALIDATIONS**
Identification tests area intended to ensure the identity of an analyte in a sample. This is normally achieved by comparison of a property of the sample to that of a reference standard.	Specificity
Impurities Quantitation are intended to accuately reflect the purity characteristic of the sample. Different validation characteristics are required for a quantitative test than for a limit test.	Accuracy, Precision, Specificity, Detection limit, Quantitation limit, Linearity, Range.
Impurites limit are intended to reflect the purity characteristics of the sample	Specificity, Detection limit
Content/ Potency; Dissolution are intended to measure the analyte present in a given sample. A quantitative measurement of the major components in the drug substance.	Accuracy, Precision, Specificity, Linearity, Range.

In the ICH-2QA: Text on Validation Analytical procedures, validation characteristics versus type of analytical procedures are shown in following table

VALIDATION CHARACTERISTICS VERSUS TYPE OF ANALYTICAL PROCEDURES				
TEST FOR IMPURITIES				
Type of procedure	Identification	Quantitation	Limit	Dissolution Measurement (content/Potency)
Accuracy	No	Yes	No	Yes
Precision /repeatability	No	Yes	No	Yes
Intermediate Precision	No	Yes[a]	No	Yes[a]
Specificity	Yes	Yes	Yes	Yes
Detection limit	No	No[b]	Yes	No
Quantitation limit	No	Yes	No	No
Linearity	No	Yes	No	Yes
Range	No	Yes	No	Yes

a. When reproducibility is performed. Intermediate precision is not needed.

b. May be needed in some cases.

The ICHQ2B: Validation of Analytical procedures, describes the main objective of validation as the ability of an analytical procedure to demonstrates that the procedure is suitable for its intended purpose. The document stresses that well-characterized reference materials, with documented purity, should be used throughout the validation study.

The comparison of different official guidelines in case of parameters required to be validated for different assays is shown in following table

COMPARATIVE TABLE REPRESENTING FDA, USP AND ICH REQUIREMENTS

CRITERIA	GMP	FDA	USP	ICH
Accuracy	X	X	X	X
Precision		X	X	X
Specificity	X	X	X	X
Detection limit			X	X
Quantitation limit			X	X
Linearity		X	X	X
Range			X	X
Reproducibility	X			X
Sensitivity	X			
Recovery		X		
Ruggedness		X	X	

LITERATURE REVIEW

Yanamandra R ***et al***[20]., developed a new rapid, simple, sensitive, selective and accurate reversed-phase stability-indicating Ultra Performance Liquid Chromatography (RP-UPLC) technique for the assay of Tolterodine Tartrate in pharmaceutical dosage form, human plasma and urine samples. The developed UPLC method is superior in technology to conventional HPLC with respect to speed, solvent consumption, resolution and cost of analysis. Chromatographic run time was 6 min in reversed-phase mode and ultraviolet detection was carried out at 220 nm for quantification. Efficient separation was achieved for all the degradants of Tolterodine Tartrate on BEH C18 sub-2-µm Acquity UPLC column using Trifluoroacetic acid and acetonitrile as organic solvent in a linear gradient program. The active pharmaceutical ingredient was extracted from tablet dosage form using a mixture of acetonitrile and water as diluent. The calibration graphs were linear and the method showed excellent recoveries for bulk and tablet dosage form. The test solution was found to be stable for 40 days when stored in the refrigerator between 2 and 8 °C. The developed UPLC method was validated and meets the requirements delineated by the International Conference on Harmonization (ICH) guidelines with respect to linearity, accuracy, precision, specificity and robustness. The intra-day and inter-day variation was found be less than 1%. The method was reproducible and selective for the estimation of Tolterodine Tartrate. Because the method could effectively separate the drug from its degradation products, it can be employed as a stability-indicating one.

Dwibhashyam VS ***et al***[21]., developed a selective, sensitive, and rugged reverse phase-high performance liquid chromatographic method for the determination of Tolterodine tartrate in routine quality control samples. The mobile phase consisted of acetonitrile:phosphate buffer (pH 7.0) in 55:45 v/v ratio. The mobile phase was also used for the extraction of tolterodine tartrate from its formulations. The chromatography was carried out on a Luna 100A, C-18 (5-micro, 250 x 4.60 mm) column. The software used in the chromatographic analysis was Empower Photodiode Array (PDA) software (Waters, Milford, CT). The UV spectrophotometric determination was done at 210 nm. Retention time was found to be about 7.0 +/- 0.5 min. The standard curve was linear (r2 = 0.9997) over the concentration range of 0.1-0.3 mg/mL. The method was found to be accurate, precise, specific, and rugged. The limit of detection was 0.16 microg/mL and the limit of quantification was 0.489 microg/mL.

With a short chromatographic run time, the proposed method can be used for the estimation of large number of quality control samples in a short period.

Xia ZL *et al*[22].,developed a high-performance liquid chromatographic method for the separation of the enantiomers of tolterodine tartarate. The proposed method was applied to the determination of (S)-isomer in (R)-tolterodine tartarate, and satisfactory results were obtained. The enantiomers of tolterodine tartarate were separated on a Chiralpak AD-H (250 mm x 4.6 mm) column containing amylase tris-(3,5-dimethylphenyl-carbamate) at room temperature. The mobile phase consisted of n-hexane and isopropyl alcohol in the ratio of 85:15 (v/v) with 0.075% triethylamine (TEA) and 0.05% trifluoroacetic acid (TFA) as the additive. The flow rate was kept at 0.5 ml/min, and UV detection wavelength was set at 283 nm. The calibration curves of (S)-enantiomer in the concentration range from 0.05 microg/ml to 1 microg/ml range were linear. The relative standard deviations of within-day and between-day were less than 2% (n = 3). The limit of detection (LOD) was 0.75 ng (S/N = 3) and the limit of quantification (LOQ) was 0.05 microg/ml (RSD < 4.1%, n = 3). The determination recoveries of the (S)-enantiomer were in the range of 98.2-104.8%. The results demonstrated that the developed HPLC method was a reliable, simple technique and was applicable to the purity determination of (R)- tolterodine tartarate.

Guan Y *et al*[23].,developed and validated a sensitive, selective and efficient liquid chromatographic/tandem mass spectrometric (LC/MS/MS) method for the determination of glucosamine in healthy human urine. Urine samples were extracted by acetonitrile and derivatized with o-phthalaldehyde/3-mercaptopropionic acid. Analysis was then carried out using ESI source and methanol/0.2% ammonium acetate-0.1% formic acid mobile phase gradient elution, with tolterodine tartrate as the internal standard. The linear calibration curve ranged from 0.41μg/ml to 82.7μg/ml. The intra-day and inter-day precisions were less than 3.93% and 10.0%, respectively. The extraction recoveries determined at three concentration levels were higher than 88.6%. The method was successfully applied for determining the urine concentration of glucosamine up to 24h after oral administration of 1g glucosamine sulfate dispersible table (containing 785.08mg glucosamine) from a clinical pharmacokinetic study in healthy volunteers.

Macek J *et al*[24].,developed a rapid and reliable method to quantitate tolterodine and its 5-hydroxymethyl metabolite in human plasma using liquid chromatography-electrospray tandem mass spectrometry. The assay was based on liquid-liquid extraction of the compounds from plasma with tert-butylmethylether and hydrophilic interaction

chromatography performed on a silica column (30mmx4.6mm, 3microm particles), the mobile phase consisted of acetonitrile-20mM ammonium acetate (70:30, v/v). Quantification was through positive-ion m` ode and selected reaction monitoring at m/z 326-->147 for tolterodine, 342-->223 for the 5-hydroxymethyl metabolite and 260-->183 for the internal standard propranolol, respectively. The lower limit of quantitation was 49 and 46pg/ml using 0.5ml of plasma for the parent drug and its metabolite, respectively and linearity was observed up to 30ng/ml. Within-day and between-day precision expressed by relative standard deviation was less than 11% and inaccuracy did not exceed 7% at all levels. The assay was applied to the analysis of samples from a pharmacokinetic study.

Xia ZL *et al*[25].,developed a high-performance liquid chromatographic method for the separation of the enantiomers of tolterodine tartarate. The proposed method was applied to the determination of (S)-isomer in (R)-tolterodine tartarate, and satisfactory results were obtained. The enantiomers of tolterodine tartarate were separated on a Chiralpak AD-H (250 mm x 4.6 mm) column containing amylase tris-(3,5-dimethylphenyl-carbamate) at room temperature. The mobile phase consisted of n-hexane and isopropyl alcohol in the ratio of 85:15 (v/v) with 0.075% triethylamine (TEA) and 0.05% trifluoroacetic acid (TFA) as the additive. The flow rate was kept at 0.5 ml/min, and UV detection wavelength was set at 283 nm. The calibration curves of (S)-enantiomer in the concentration range from 0.05 microg/ml to 1 microg/ml range were linear. The relative standard deviations of within-day and between-day were less than 2% (n = 3). The limit of detection (LOD) was 0.75 ng (S/N = 3) and the limit of quantification (LOQ) was 0.05 microg/ml (RSD < 4.1%, n = 3). The determination recoveries of the (S)-enantiomer were in the range of 98.2-104.8%. The results demonstrated that the developed HPLC method was a reliable, simple technique and was applicable to the purity determination of (R)- tolterodine tartarate.

Böttiger Y *et al*[26].,studied about the hydroxylation of omeprazole and measured as the ratio of omeprazole/5-hydroxyomeprazole in a plasma sample taken 3 h after an oral dose,and claimed that it is an established method to determine CYP2C19 activity, and the ratio of omeprazole AUC/omeprazole sulfone AUC has been used for assessing CYP3A4 activity. The aim of this study was to determine whether the latter ratio from a single 3-h sample can also be used for CYP3A4 phenotyping. Plasma levels of omeprazole and omeprazole sulfone were analyzed by reversed-phase high-performance liquid chromatography in a blood sample drawn 3 h after intake of a single oral 20-mg dose of omeprazole by 22 healthy subjects and five patients with newly diagnosed epilepsy. The procedure was repeated on the 4th day of 200 mg of ketoconazole intake (10 subjects), after 3 weeks of 150-200 mg twice-daily

carbamazepine (five patients), and on the 6th day of 4 mg twice-daily tolterodine (12 subjects). Five subjects also took 100 mg and 50 mg of ketoconazole for 3 days before concomitant intake with omeprazole. The mean log10(omeprazole/omeprazole sulfone) ratio was 0.18 3 h after intake of omeprazole alone. After concomitant intake of ketoconazole, the corresponding value was 1.38 ($p<0.001$); after intake of carbamazepine it was -0.42 ($p<0.05$); and after tolterodine it was 0.29 (not significant). In the five subjects taking increasing doses of ketoconazole, the ratio was 0.11, 0.79, 1.2, and 1.5 after 0, 50, 100, and 200 mg of ketoconazole, respectively. The correlation between the metabolic ratios from the AUC((0-6h)) and from the single 3-h samples was very good, with a correlation coefficient of 0.92 ($p<0.001$). A single blood sample taken 3 h after intake of 20 mg of omeprazole can be reliably used to phenotype for both CYP2C19 and CYP3A4 activity.

Zhang B *et al*[27].,developed a selective and sensitive high performance liquid chromatography-electrospray ionization mass spectrometry method for the determination of tolterodine tartrate in human plasma. With oxybutynin as internal standard, tolterodine tartrate was extracted from plasma with n-hexane: isopropanol (95:5, v/v). The organic layer was evaporated and the residue was redissolved in mobile phase comprised of acetonitrile-water (10 mM CH3COONH4, pH 3.0)=50:50 (v/v). An aliquot of 10 microl was chromatographically analyzed on a prepacked Shimadzu Shim-pack VP-ODS C18 column (150 mmx2.0 mm I.D.) by means of selected-ion monitoring (SIM) mode mass spectrometry. Standard curves were linear ($r=0.9993$) over the concentration range of 0.1-30.0 ng/ml and had good accuracy and precision. The within- and between-batch precisions were within 10% relative standard deviation. The limit of detection (LOD) was 0.05 ng/ml. The validated LC-ESI-MS method has been used successfully to study tolterodine tartrate pharmacokinetic, bioavailability and bioequivalence in 20 healthy male volunteers.

Hosseinpour F *et al*[28].,carried a work on the following ,Porcine CYP2D25, microsomal vitamin D(3) 25-hydroxylase, catalyzes the essential first step in the bioactivation of the prohormone vitamin D(3). Although CYP2D25 shows a high degree of sequence identity with other members of the CYP2D subfamily, such as human CYP2D6, the vitamin D(3) 25-hydroxylase activity is a unique property among CYP2D enzymes. In addition to 25-hydroxylation, CYP2D25 also metabolizes the drug tolterodine. In this study, CYP2D25 was functionally expressed in the Saccharomyces cerevisiae W(R) strain and site-directed mutagenesis was used to study the role of substrate recognition site 3 (SRS-3) for the catalytic specificity of CYP2D25. Five residues in SRS-3 of CYP2D25 were simultaneously

mutated to the equivalent residues in CYP2D6, an enzyme not active in 25-hydroxylation. Western blot analysis of microsomes from transformed yeast cells showed that both the wild-type and mutant CYP2D25 were expressed at comparable levels. The 25-hydroxylase activity of recombinant mutant CYP2D25 was completely lost whereas the activity toward tolterodine remained virtually unaffected. The results implicate that residues in SRS-3 of CYP2D25 are important determinants for its function in vitamin D(3) metabolism.

Swart R *et al*[29].,developed a method where a capillary solid-phase extraction (SPE) system has been coupled directly to electrospray tandem mass spectrometry for quantification of free tolterodine and metabolite concentrations in plasma. The unbound fraction of these compounds was obtained by ultrafiltration of plasma. The ultrafiltrate was directly injected onto the SPE capillary (4 mm x 200 microm, 5 microm C18). After desalting and clean-up of the sample, the analytes were eluted in backflush mode with methanol-1 mM triethylamine (70:30, v/v), providing considerable solute focusing. Elution from the SPE capillary was improved by inserting a short trapping capillary between the SPE capillary and the MS interface, by which analyte focusing was increased. The unresolved compounds eluted simultaneously with the remaining matrix compounds and were detected in a multiple-reaction monitoring (MRM) mode. No interference of the sample matrix on detection was observed,allowing aqueous standards to be used for calibration. Linear calibration curves were obtained between 0.05 and 1000 ng/ml (corresponding to 150 pM-3 microM) and the limit of detection was 50 pg/ml injecting 10 microl. Equilibration of the SPE capillary, sample loading, elution and detection took less then 6 min per sample.

AIM AND OBJECTIVE OF WORK

Literature review reveals that few methods have been published for analysis of Tolterodine tartrate tablets in the biological fluids and samples. But the methods are somewhat costlier as they are using costlier solvents and the detectors used by them are mostly PDA detectors. So, the objective of this work was to develop and validate an isocratic RP-HPLC method for quantitative analysis of Tolterodine tartrate in tablet dosage form which is very easy for the analyst to analyse and by using the economic solvents with UV detector as this detector is commonly available detector.

PLAN OF WORK

Method Development Parameters Done By RP-HPLC

- Selection of detector wavelength
- Selection of column
- Selection of mobile phase composition and pH
- Selection of flow rate

Validation of the Developed Method

a) Linearity
b) System precision
c) Method precision
d) Specificity
e) Accuracy
f) Ruggedness
g) Robustness
h) Limit of detection and quantification
i) Solution stability
j) System suitability

DRUG PROFILE

Tolterodine tartrate

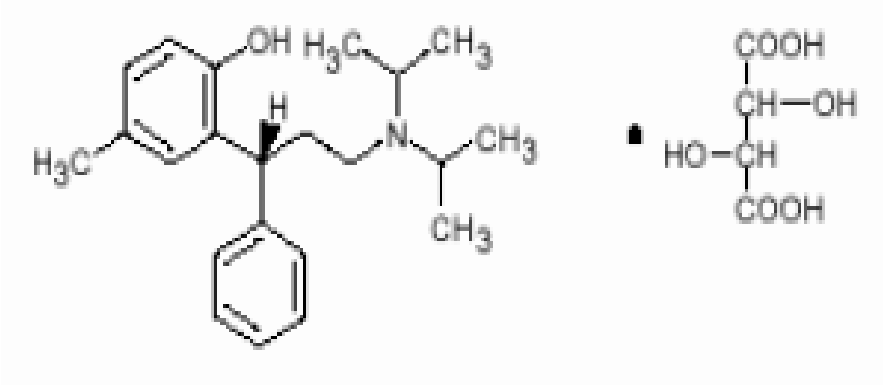
Description : White-to-Off-White Crystalline Powder

IUPAC Name : (R)-2-(3-(Bis(1-methylethyl)amino)-1-phenylpropyl)-4-methylphenol (R-(R',R'))-2,3-dihydroxy butanedioate

Synonym : (+)-R)-2-{a-[2-(Diisopropylamino)ethyl]benzyl}-p-cresol tartrate

Chemical Formula : $C_{26}H_{37}NO_7$

Chemical Structure :



Molecular Weight : 475.6

Melting Point : 205-210°C

Solubility : The solubility in water is 12 mg/mL. It is soluble in methanol, slightly soluble in ethanol, and practically insoluble in toluene.

Category : A muscarinic receptor antagonist. Used in the treatment of urinary incontinence.

INSTRUMENTS AND REAGENTS

Instruments

Sl.No	Name of the instrument	Make	Model
1	UV-VIS Double beam Spectrophotometer	Shimadzu	AL-2401
2	HPLC-UV	Agilent	LC-2011
3	Millipore	Millipore	Simplicity
4	Electronic balance	Shimadzu	AD220D

Reagents and Chemicals

S.NO	Name	Grade	Manufacturer/Supplier
1	ACN	HPLC	MERCK
2	Di sodium hydrogen Phosphate	HPLC	MERCK
3	Millipore water	-	-
4	Phosphoric acid	HPLC	MERCK

Standard

The reference standard Tolterodine tartrate was obtained as gift sample and authenticity and purity of the sample was certified by Ranbaxy.

Formulation

Label Claim : 1mg

Brand Name : Roliten

Company Name : Ranbaxy

METHOD DEVELOPMENT AND OPTIMIZATION

1. Selection of wavelength

The known concentration of Tolterodine tartrate was taken and dissolved in water. The resulting solution was then scanned between 200 to 400 nm and is shown in fig 1.Maximum absorbance was found at 302nm and it was selected for the analysis of Tolterodine tartrate.

2. Optimization of Chromatographic Parameters

a. Selection of mode of operation

As the drug was polar in nature, RP-HPLC was preferred.

b. Selection of mobile phase

The method development of Tolterodine tartrate required adequate resolution of the drug peak in the chromatogram. Different solvent systems were tried to get the proper resolution

3. Preparation of mobile phase

Acetonitrile and Di sodium hydrogen phosphate in the ratio 27:73 was taken and the pH of the mobile phase was maintained at 7.6 with phosphoric acid and it was filtered. Then it was degassed.

4. Selection of flow rate

The flow rate for Tolterodine tartrate was tried at different rates. Finally 1ml/min was selected at which the peak was free from any fronting or tailing effects.

Preparation of Phosphate Buffer (pH 7.6):

Place 50 ml of 0.2M potassium dihydrogen phosphate in a 200ml volumetric flask, add 42.4ml of 0.2M sodium hydroxide and then add water to volume.

5. Determination of retention time

Standard solution of Tolterodine tartrate:

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark

with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

20µl of the solution was injected and the chromatogram was recorded and it is shown in fig 3.

FIXED CHROMATOGRAPHIC CONDITIONS

Instrument	: AGILENT LC-2011with UV-VIS Detector
Column	: C_{18}
Wavelength	: 302nm
Flow rate	: 1ml/min
Injection	: 20µl
Mobile Phase	: Buffer: ACN (73:27) pH maintained at 7.6 with phosphoric acid
Retention time	: 12.994 mins

QUANTITATIVE ESTIMATION OF THE DRUG

Sample : Tolterodine tartrate

Label claim : 1mg

Standard solution of Tolterodine tartrate

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Sample preparation

20 Tolterodine tartrate tablets were randomly selected, weighed and their average weight was calculated. Then the sample equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. Diluent was added and then the volume was made up to the mark. The solution was then shaken well to dissolve the contents and then filtered.10ml of the filtrate was diluted to 100ml with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

The amount of Tolterodine tartrate present in the tablet formulation was calculated by comparing the peak area of the standard and sample which is given in fig 5 & 6. The reports are given in table 1.

Amount of drug present in the tablet =

$$\frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \frac{\text{Potency}}{100} \times \text{Average weight of tablet}$$

Amount present

Percentage content = ---------- x 100
Label claim

VALIDATION

Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Design of experiment:

Typical analytical parameters used in assay validation are,

- Specificity
- Linearity and range
- Limit of quantification
- Limit of detection
- Accuracy
- Precision
    - System precision
    - Method precision
- Robustness
- Ruggedness
- System suitability studies
    - Resolution
    - Number of theoretical plates
    - The tailing factor

LINEARITY AND RANGE

Linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range.

Determination:

The linearity of the analytical method for the assay was demonstrated by injecting the different concentrations of the standard preparation in the range of 10-100% into the chromatograph, covering 10 different concentrations. A plot was drawn between the concentration vs peak response of Tolterodine tartrate. The slope, intercept and regression coefficient from the plot obtained for concentration vs peak response of Tolterodine tartrate was reported.

Method:

Preparation of standard solution

100mg of Tolterodine tartrate standard was transferred into 100ml volumetric flask and diluent was added. The volume was then made up with diluent. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. From the resulting solution 0.2,0.4,0.6,0.8, 1,1.2,1.4,1.6,1.8 and 2 were transferred to 10 different 10ml volumetric flasks and the volumes were made up with diluent. The solutions were then filtered through 0.45µ membrane filters.

$20\,\mu l$ of the resulting solutions were injected and chromatograms were recorded. The chromatograms of Tolterodine tartrate are shown in figure 7-16.

The correlation coefficient and percentage curve fitting were calculated from the following formula,

$$R = \sum \frac{(X - \bar{X})(y - \bar{y})}{n - 1\, S_x\, S_y}$$

Where, x = concentration, y = instrumental response, S_x = Std. Deviation of x S_y = Std. Deviation of y

Percentage curve fitting = 100x correlation coefficient

Acceptance Criteria

- Correlation coefficient should not be less than 0.97%
- Curve fitting should not be less than 99.7%

The linearity Data and analytical performance parameters of Tolterodine tartrate is shown in Table 2-3 and calibration curve of Tolterodine tartrate is shown fig 17.

PRECISION

Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation.

SYSTEM PRECISION

The system precision was evaluated by measuring the peak response of drug for six replicate injections of the standard solution as per the proposed method.

Acceptance criteria: The relative standard deviation for the six standard preparations of the same batch should not be more than 2.0%

Blank solution

Mixture of Buffer and ACN in the ratio 73:27 was taken and degassed.

Standard solution of Tolterodine tartrate

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

In the same manner five other standard solutions were also prepared. 20µl of the working standards were injected. The peak responses were measured from the chromatograms shown in fig 18-23and system precision data are shown in table 4.

The standard deviation and relative standard deviation were calculated from the statistical formula

$$\text{Standard deviation} = \sigma \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}}$$

x = sample, $\bar{x}$ = mean value of sample, n = number of sample

$$\text{Relative standard deviation} = \frac{\sigma}{\bar{x}} x100$$

METHOD PRECISION

The method precision was determined by preparing the sample of single batch of Tolterodine tartrate tablets for six times and analysed as per the proposed method.

Acceptance criteria: The relative standard deviation for the assay values of six sample preparations of the same batch should be not more than 2.0%

Blank solution

Mixture of Buffer and ACN in the ratio 73:27 was taken and degassed.

Sample preparation

20 Tolterodine tartrate tablets were randomly selected, weighed and their average weight was calculated. Then the sample equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. Diluent was added and then the volume was made up to the mark. The solution was then shaken well to dissolve the contents and then filtered.10ml of the filtrate was diluted to 100ml with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter. The procedure was repeated another five times to give a set of 6 replicate solutions.

20 µl of working sample solution were injected and the chromatograms were recorded and shown in fig 24-29 and method precision data are shown in table 5.

The standard deviation and relative standard deviation were calculated from the statistical formula

$$\text{Standard deviation} = \sigma \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

x = sample, $\bar{x}$ = mean value of sample, n = number of sample

$$\text{Relative standard deviation} = \frac{\sigma}{\bar{x}} x100$$

SPECIFICITY

The specificity of an analytical method is its ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix

Determination

The specificity of the analytical method was determined by injecting the placebo solution under the same experimental conditions as the assay.

Preparation of placebo

Placebo was prepared by mixing all the excipients other than the active ingredients.

Procedure

About 8.5gm of placebo was accurately weighed and transferred to a 100ml volumetric flask. Then diluent was added and the volume was made up to the mark. The solution was then filtered through Whatmann filter paper. 10ml of the above solution was pipetted into a 100ml volumetric flask and the volume was made up with millipore water. From the resulting solution 1ml was taken and made upto 10ml. The solution was then filtered through 0.45µ membrane filter.

20µl of the solution was injected and the chromatogram was recorded which is shown in fig 31.

Preparation of Tolterodine tartrate standard

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from

the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

20µl of the solution was injected and the chromatogram was recorded which is shown in fig 32.

Preparation of standard + placebo

100mg of accurately weighed standard and 8.5gm of placebo was transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. From the resulting solution 1ml was pipetted out and made upto 10ml with diluent. The solution was then filtered through 0.45µ membrane filter.

20µl of the solution was injected and the chromatogram was recorded which is shown in fig 33 and the data values are given in table 6

ACCURACY

The accuracy of an analytical method is the closeness of test results obtained by that method to the true value. Accuracy may often the expressed as percent recovery by the assay of known added amounts of analyte.

Determination:

The accuracy of the analytical method is determined by applying the method to analyzed samples, to which known amounts of analyte have been added. The accuracy is calculated from the test results as the percentage of analyte recovered by the assay.

Acceptance Criteria: Percentage recovery should be within 98-102%

Procedure:

Different concentrations of Tolterodine tartrate were prepared by taking different weights and dissolved in diluent. From the resulting solutions, suitable dilutions are made to give concentrations of 80%, 100% and 120%. The peak areas of the different concentration of solutions are then determined in triplicates, as shown in fig 34-42 and values are given in table 7.The amount is calculated using the equation

$$\text{Accuracy} = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Standard dilution}}{\text{Sample dilution}} \times \text{Potency}$$

RUGGEDNESS

The ruggedness of an analytical method is degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions, such as different laboratories, different analysts, different instruments, different lots of reagents, different elapsed assay times, different assay temperatures, different days, etc.

Ruggedness is normally expressed as a lack of influence on test results of operational and environmental variables of the analytical method.

Procedure

Standard solution of Tolterodine tartrate

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Sample preparation

20 Tolterodine tartrate tablets were randomly selected, weighed and their average weight was calculated. Then the sample equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. Diluent was added and then the volume was made up to the mark. The solution was then shaken well to dissolve the contents and then filtered.10ml of the filtrate was diluted to 100ml with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Method:

The standard stock solution and sample stock solution were prepared by different analysts on different days & different instruments and the resulting solutions were injected and the chromatograms were recorded which is shown in fig 43-62. The ruggedness of the method and report of the method is shown in table 8-10.

ROBUSTNESS

Robustness of an analytical method is the measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

Determination:

The robustness of an analytical method was determined by analysis of aliquots from homogenous lots by differing physical parameters that may differ but were still within the specified parameter of the assay for example change in physical parameters like flow rate and mobile phase ratio.

Acceptance Criteria:

The %RSD for the assay values of Tolterodine tartrate in Roliten tablets 1mg obtained under deliberately modified chromatographic conditions should not be more than 2.0%. The difference between the assay under the modified conditions and the assay obtained under precision should not be more than 2.0%

Method:

Preparation of blank

Mixture of Buffer and ACN in the ratio 73:27 was taken and degassed.

Preparation of standard solution

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Preparation of sample solution

20 Tolterodine tartrate tablets were randomly selected, weighed and their average weight was calculated. Then the sample equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. Diluent was added and then the volume was made up to the mark. The solution was then shaken well to dissolve the contents and then filtered.10ml of the filtrate was diluted to 100ml with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Procedure

20µl of blank, standard and sample preparations were injected into the chromatograph which was set under deliberately modified chromatographic conditions and the chromatograms were recorded. The graphs are shown in fig 63-86 and the results are shown in table 11-18.

SOLUTION STABILITY

To establish the stability of analytical solutions the standard and sample solutions were injected at periodic intervals upto 24hrs.

Acceptance criteria: The %RSD of peak response for the major peak of both standard and sample solutions at periodic intervals should not be more than 2.0%

Blank Solution

Mixture of Buffer and ACN in the ratio 73:27 was taken and degassed.

Preparation of standard solution

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Preparation of sample solution

20 Tolterodine tartrate tablets were randomly selected, weighed and their average weight was calculated. Then the sample equivalent to 100mg was weighed and transferred into a 100ml volumetric flask. Diluent was added and then the volume was made up to the mark. The solution was then shaken well to dissolve the contents and then filtered.10ml of the filtrate was diluted to 100ml with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Procedure

20µl of blank, standard and sample preparations were injected separately into the chromatograph and the peak responses were measured for the major peak and they are shown in Fig87-122 and the data values are given in table 19

LIMIT OF DETECTION

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantified as an exact value under the stated, experimental conditions. Several approaches for determining the detection limit are possible, depending on whether the procedure is non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

1. Based on visual evaluation
2. Based on signal-to-noise.
3. Based on the standard deviation of the Response and slope.

Here the calculations were made on the basis of standard deviation of the response and slope.

Based on the standard deviation of the Response and slope:

A specific calibration curve was studied using samples containing an analyte in the range of detection limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The detection limit may be expressed as:

$$\mathrm{LOD} = \frac{3.3\sigma}{S}$$

Where, σ = standard deviation of the response

S=slope of the calibration curve

The slope S was estimated from the calibration curve of the analyte.

The data are given in table 20.

LIMIT OF QUANTITATION

It is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Several approaches for determining the quantitation limit are possible, depending on whether the procedure is a non-instrumental or instrumental. Approaches other than those listed below may be acceptable.

1. Based on visual evaluation
2. Based on signal-to-noise.
3. Based on the standard deviation of the Response and slope.

Here the calculations were made on the basis of the standard deviation of the response and slope.

Based on the standard deviation of the Response and slope:

A specific calibration curve was studied using samples containing the analyte in the range of quantitation limit. The residual standard deviation of a regression line or the standard deviation of y-intercepts of regression lines may be used as the standard deviation.

The quantitation limit may be expressed as:

$$LOQ = \frac{10\sigma}{S}$$

Where, σ = standard deviation of the response

S=slope of the calibration curve

The slope S may be estimated from the calibration curve of the analyte.

The data are given in table 21.

SYSTEM SUITABILITY

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operation and sample to be analysed constitute an integral system that can be evaluated as such. System suitability test parameters to be established for a particular procedure depend on the type of procedure being validated.

Acceptance Criteria:

- The column efficiency is not less than 2000 theoretical plates
- The tailing factor for the analyte peak is not more than 2.0
- The relative standard deviation for the replicate injection is not more than 2.0%

Determination

To determine the suitability of chromatographic system described for the method of analysis by establishing system suitability parameters like peak tailing factor, number of theoretical plates and the %RSD of Tolterodine standard preparation on daily basis

Preparation of standard solution

100mg of Tolterodine tartrate standard was accurately weighed and transferred into a 100ml volumetric flask. Then diluent was added and the volume was made upto the mark. From the stock solution 10ml was pipetted out into a 100ml volumetric flask and the volume was made up to the mark with diluent. Then from the resulting solution 1ml was taken and made upto 10ml with diluents. The solution was then filtered through 0.45µ membrane filter.

Procedure

20 µl of standard solutions were injected and chromatograms were recorded and they are shown in fig 123-128 and system suitability reports are given table 22

RESULTS AND DISCUSSION

The developed method is a simple method which has the advantage of determination of Tolterodine tartrate with simple solvent system. The analysis time is 12.994mins. This provides shorter analysis time and conserves the solvent system.

FIXED CHROMATOGRAPHIC CONDITIONS

Instrument : AGILENT LC-2011

Column : C_{18}

Wavelength : 302nm

Flow rate : 1ml/min

Injection : 20µl

Mobile Phase : Disodium hydrogen Phosphate Buffer : ACN (73:27) pH maintained at 7.6 with phosphoric acid

Retention time : 12.994 mins

Table:23 QUANTIATIVE ESTIMATION

Sl.No	Content	Standard Peak Area	Sample Peak Area	Amount present(mg)	Percentage content(%)
1	Tolterodine tartrate	30600.4623	30586.1649	0.9995	99.95

The method was validated based on USP and ICH parameters:

Parameters validated for the developed method include accuracy, linearity, precision, interference studies, detection limit, quantitation limit, stability.

Specificity of the method was found out through non-interference of the placebo in identical conditions of assay. This confirms the specificity of the developed method. Linearity of the drug was obtained in the range of 10-100µg/ml. The linearity coefficient and percentage curve fitting slope was found to be 0.999 and 99.9%.

Detection limit was done by calibration curve method.The limit of detection was found to be 0.0002241. Quantitation limit was done by calibration curve method. The limit of quantification was found to be 0.0006792.

Accuracy of the method was determined through recovery studies of the drug. Recovery of the drug is well within acceptance limits (98 to 102%). Precision of the method was determined by assays of drug formulations by replicate injection and precision of system was determined by using standard solution. %RSD of the assays is found to be within the limits of 2%. Thus the developed method is found to provide high degree of precision and reproducibility.

Ruggedness was determined by performing the same assay on different days, assay being carried out by different analysts. The test results were within the limits 98 to 102%. The result is found to be reproducible.

Robustness was determined by carrying out the assay during which the mobile phase ratio and flow rate was altered. Percent recovery was found to be within 98- 101%, which is well within the acceptable limits. The low values of RSD obtained with the change in mobile phase ratio makes it possible to carry out the method with small variations in the ratio of the mobile phase and flow rate. This indicates the lack of influence on test results by operational and environmental variables for the developed method.

Stability of the method was determined by assays of the drug formulation up to 24hrs hours. There was almost no appreciable change in absorbance up to 24hrs. Percentage recovery of the assays was found to be within limits. Thus the developed method was found to provide high degree of stability

System suitability was determined by performing the assay with the same sample repeatedly. The number of theoretical plates was found to be 4968.The tailing factor was found to be 1.2. It indicates good and complete separation of the two components from each other with well defined base line.

CONCLUSION

The developed chromatographic method for the determination of test procedure of assay for Tolterodine tartrate in pharmaceutical dosage forms were simple, reliable, sensitive and less time consuming. The advantage of the present test procedures was that it does not require any complicated mobile phase and it is simple isocratic method. The present method can be confidently used for rapid and precise quantitation of Tolterodine tartrate. This procedure can be a major interest in analytical pharmacy, since it offers a distinct quality control in the test procedure of assay of pharmaceutical dosage forms. However taking into account of the factors such as economy and rapidity, the present method can also be a good choice for the analysis of Tolterodine tartrate. The developed method may be recommended for routine and quality control analysis of the investigated drug.

The present work shows, a validated, highly sensitive and selected method for the determination of Tolterodine tartrate in pharmaceutical dosage forms. Precision and accuracy as major control parameters of the whole validation procedure was within the acceptable limits. The experimental data makes a relevant contribution to the understanding of validation parameters.

Overall the above developed analytical method represents a valuable data for the validation of Tolterodine tartrate tablets and rapid quantitation method for the same in the pharmaceutical dosage forms.

Table :1 Quantitative Estimation

Sl.No	Content	Standard Peak Area	Sample Peak Area	Amount present(mg)	Percentage content (%)
1	Tolterodine tartrate 1mg	30600.4623	30586.1649	0.9995	99.95

Table :2 Linearity Data

Sl.No	Concentration (%)	Concentration(µg/ml)	Peak Area
1	10	2	6120.0205
2	20	4	12240.1298
3	30	6	18360.4282
4	40	8	24480.1923
5	50	10	30600.1028
6	60	12	36720.2923
7	70	14	42840.2968
8	80	16	48960.3640
9	90	18	55080.2343
10	100	20	61200.3421

Table:3 Analytical Performance Parameters

Sl.No	Drug	Linearity Range	Correlation Coefficient	Percentage Curve Fitting (%)	Slope
1	Tolterodine tartrate 1mg	10-100	0.999	99.99	6120

Table:4 System Precision data

Sl.No	Area of Tolterodine
1	30601.8416
2	30600.5142
3	30601.0012
4	30599.1643
5	30600.1942
6	30601.2398
MEAN	30600.6592
S.D	0.92995
%RSD	0.00303

Table:5 Method Precision Data

Sl.No	Area Obtained	Amount Present in Tablet (gm)	Percentage content (%)
1	30584.1965	0.00099946	99.95
2	30586.2198	0.00099942	99.94
3	30586.8129	0.00099944	99.94

4	30585.7689	0.000999447	99.94
5	30584.2925	0.000999365	99.94
6	30586.4415	0.000999435	99.94
MEAN			99.942
STANDARD DEVIATION			0.00408
%RSD			0.00408

Table:6 Specificity for Tolterodine

Sl.No	Sample	Area Obtained
1	Placebo	0
2	Standard	30600.4623
3	Standard + Placebo	30601.3146

Table no:7 Recovery study of Tolterodine

S.No	SAMPLE - ID	Amount added (mg)	Area obtained	Amount found (mg)	Percentage Recovery(%)
1.	80%	8	24480.1874	7.991	99.89
		8	24481.0210	7.992	99.90
		8	24479.9862	7.991	99.89
2.	100%	10	30600.2342	9.989	99.89
		10	30600.0102	9.989	99.89
		10	30599.8929	9.989	99.89
3.	120%	12	36720.2810	11.987	99.89
		12	36721.7829	11.988	99.90

		12	36719.9928	11.987	99.89
Mean					99.89
Standard Deviation					0.00192
% R.S.D					0.001922

RUGGEDNESS

Table:8 Analyst 1

S.No	Date of Analysis	Standard Area	Sample area	Assay Value in (gm)	Percentage content(%)
1	28.10.12	30595.6978	30588.1789	0.00099965	99.97
2	29.10.12	30599.2875	30587.9876	0.00099953	99.95
	MEAN				99.96
	STANDARD DEVIATION				0.01414
	% RELATIVE STANDARD DEVIATION				0.01414

Table:9 Analyst 2

S.No	Date of	Standard	Sample area	Assay Value	Percentage

	Analysis	Area		in (gm)	content(%)
1	28.10.12	30600.4253	30587.2349	0.00099947	99.95
2	29.10.12	30598.2395	30586.7279	0.00099952	99.95
	MEAN				99.95
	STANDARD DEVIATION				0.01414
	% RELATIVE STANDARD DEVIATION				0.01414

Table:10 Instrument variation

S.No	Instrument	Standard Area	Sample Area	Assay Value in (gm)	Percentage content(%)	
1	INSTRUMENT I	30599.6787	30586.4575	0.000999468	99.95	
		30597.2686	30588.2939	0.000999607	99.96	
		30596.3525	30584.3292	0.000999507	99.95	
2	INSTRUMENT II	30597.9982	30581.9982	0.000999377	99.94	
		30598.2212	30586.2343	0.000999508	99.95	
		30599.6525	30587.9324	0.000999517	99.95	
	MEAN				99.95	99.95
	STANDARD DEVIATION				0.00577	0.00577
	% RELATIVE STANDARD DEVIATION				0.00577	0.00577

Table:11

CHROMATOGRAPHIC CONDITION : CHANGE IN MOBILE PHASE RATIO

BUFFER :ACN(74:26)

Flow rate	1.0 ml/min
Instrument	LC-2011
Column	C_{18}
Wavelength	302nm
Injection volume	20µl
Mobile phase	BUFFER :ACN(74:26)

Table:12 Change In Mobile Phase Ratio

Sl.No	Drug	Average standard area	Average sample area	Percentage content (%)
1	Tolterodine tartrate	30605.1663	30586.9790	99.93

Table:13

CHROMATOGRAPHIC CONDITION : CHANGE IN MOBILE PHASE RATIO

BUFFER :ACN(72:28)

Flow rate	1.0ml/min
Instrument	LC-2011
Column	C_{18}
Wavelength	302nm
Injection volume	20µl
Mobile phase	BUFFER :ACN(72:28)

Table :14 Change in mobile phase ratio

Sl.No	Drug	Average standard area	Average sample area	Percentage content (%)
1	Tolterodine tartrate	30595.1273	30581.8952	99.95

Table:15

CHROMATOGRAPHIC CONDITION : CHANGE IN FLOW RATE 1.1ml/min

Change in flow rate	1.1ml/min
Instrument	LC-2011
Column	C_{18}
Wavelength	302nm
Injection volume	20µl
Mobile phase	BUFFER :ACN(73:27)

Table:16 Change In Flow Rate 1.1ml/min

Sl.No	Drug	Average standard area	Average sample area	Percentage content(%)
1	Tolterodine tartrate	30607.0954	30587.7695	99.93

Table:17

CHROMATOGRAPHIC CONDITION : CHANGE IN FLOW RATE 0.9ml/min

Change in flow rate	0.9ml/min
Instrument	LC-2011
Column	C_{18}
Wavelength	302nm
Injection volume	20µl
Mobile phase	BUFFER :ACN(73:27)

Table:18 Change In Flow Rate 0.9ml/min

Sl.No	Drug	Average standard area	Average sample area	Percentage content(%)
1	Tolterodine tartrate	30594.7321	30582.6472	99.95

Table:19 Stability Data

Sl.No	Time Intervals(Hours)	Peak area of blank	Peak area of placebo	Peak area of Standard	Peak area of Sample
1	0	0	0	30600.2343	30585.2214
2	1	0	0	30600.2404	30586.9826
3	2	0	0	30600.6238	30586.6263

4	3	0	0	30600.9219	30584.9989
5	4	0	0	30601.0201	30585.0230
6	5	0	0	30600.4238	30586.0982
7	6	0	0	30599.9826	30585.2218
8	7	0	0	30599.6823	30585.0023
9	8	0	0	30600.0981	30586.7826
10	9	0	0	30599.8623	30586.2302
11	10	0	0	30600.3324	30586.9202
12	12	0	0	30599.5436	30586.9298
13	14	0	0	30599.1829	30585.8223
14	16	0	0	30600.1020	30586.0813
15	18	0	0	30600.0023	30585.0009
16	20	0	0	30599.2864	30586.2292
17	24	0	0	30600.3324	30586.9202
MEAN				30600.1100	30586.0054
STANDARD DEVIATION				0.50696	0.78435
% RSD				0.00165	0.00256

Table:22 SYSTEM SUITABILITY PARAMETERS

Parameters	**Results**
Tailing Factor	1.2
Theoretical Plates	4968
%RSD peaks	0.00136

Table:20 Limit Of Detection

Sl.No	Area of Tolterodine tartrate
1	30600.4620
2	30600.0021
3	30600.2212
4	30599.2235
5	30599.9829
6	30600.0123
MEAN	30599.984
S.D	0.4157
%RSD	0.00136

$$LOD = \frac{3.3\sigma}{S}$$

$$= 0.0002241$$

Table:21 Limit Of Quantitation

Sl.No	Area of Tolterodine tartrate
1	30600.4620
2	30600.0021
3	30600.2212
4	30599.2235
5	30599.9829
6	30600.0123
MEAN	30599.984
S.D	0.4157
%RSD	0.00136

$$LOQ = \frac{10\sigma}{S}$$

$$= 0.0006792$$

Table:24 VALIDATION PARAMETERS

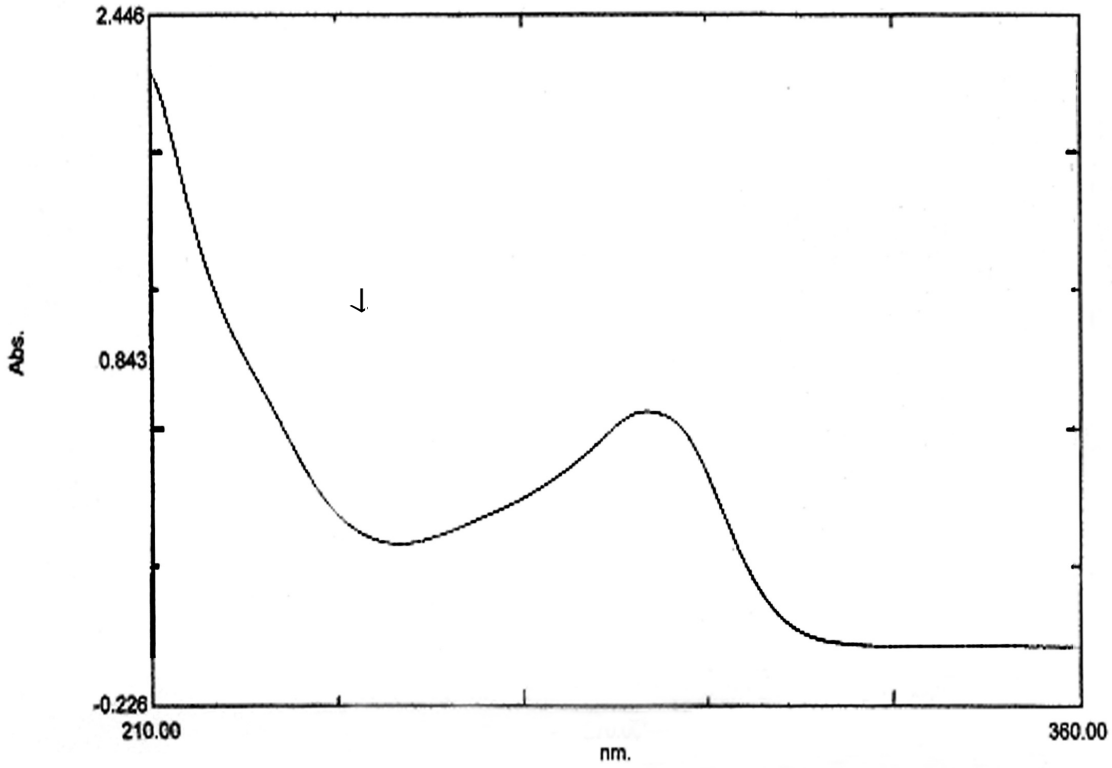
Sl.No	Parameters	Results	Acceptance Criteria
1	Specificity	Complies	Complies
2	Correlation Coefficient	0.999	0.99
3	Accuracy	99.89	98-102%
4	Precision		
	System precision	0.00303	RSD NMT 2.0%
	Method precision	0.00408	RSD NMT 2.0%
5	Ruggedness Analyst I Analyst II Instrument I Instrument II	 0.01414 0.01414 0.00577 0.00577	RSD NMT 2.0%
6	Robustness a)Change in mobile phase ratio Buffer :ACN(74:26) Buffer :ACN(75:25)	 99.93 99.95	98-102%
	b)Change in flow rate 0.9ml/min 1.1ml/min	99.93 99.95	98-102%
7	LOD	0.0002241	-
8	LOQ	0.0006792	-
9	Solution stability a)Standard response b)Sample response	0.00165 0.00256	RSD NMT 2.0% RSD NMT 2.0%
10	System suitability a)Tailing Factor b)Theoretical plates c)%RSD Peaks	1.2 4968 0.00136	NMT 2 NLT2000 RSD NMT 2.0%

Fig 1. UV Spectrum of Tolterodine tartrate

Spectrum Point Pick Report

1 / 9/2012 12:35:45 PM

Data Set: 162912 - RawData - C:\Program Files\Shimadzu\UVProbe\Data\Tolterodine tartrate



Measurement Properties

Wavelength Range (nm.):	210.00 to 360.00
Scan Speed:	Medium
Sampling Interval:	0.1
Auto Sampling Interval:	Enabled
Scan Mode:	Auto

No.	Wavelength	Absorbance	Description
1	280.00	0.735	
2	302.00	0.982	

Sample Preparation Properties

Weight:	
Volume:	
Dilution:	
Path Length:	
Additional Information:	

Instrument Properties

Instrument Type:	UV-2401
Measuring Mode:	Absorbance
Slit Width:	1.0 nm
Light Source Change Wavelength:	360.0 nm
S/R Exchange:	Normal

Attachment Properties

Attachment:	None

Fig 2: Blank(Mobile Phase)

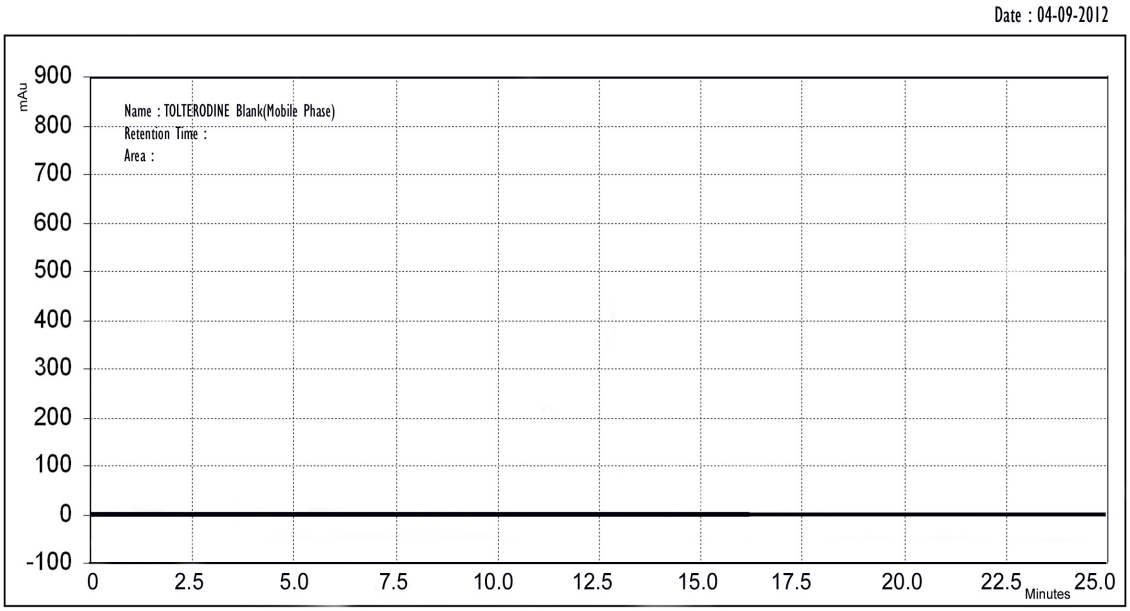


Fig 3: Standard

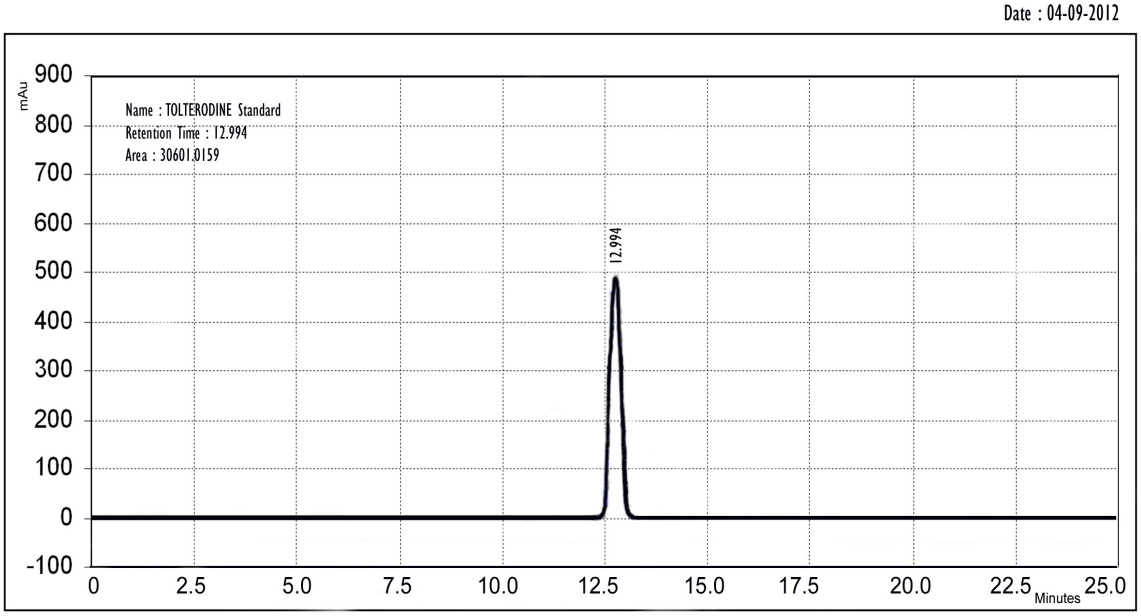


Fig 4: Blank(Mobile Phase)

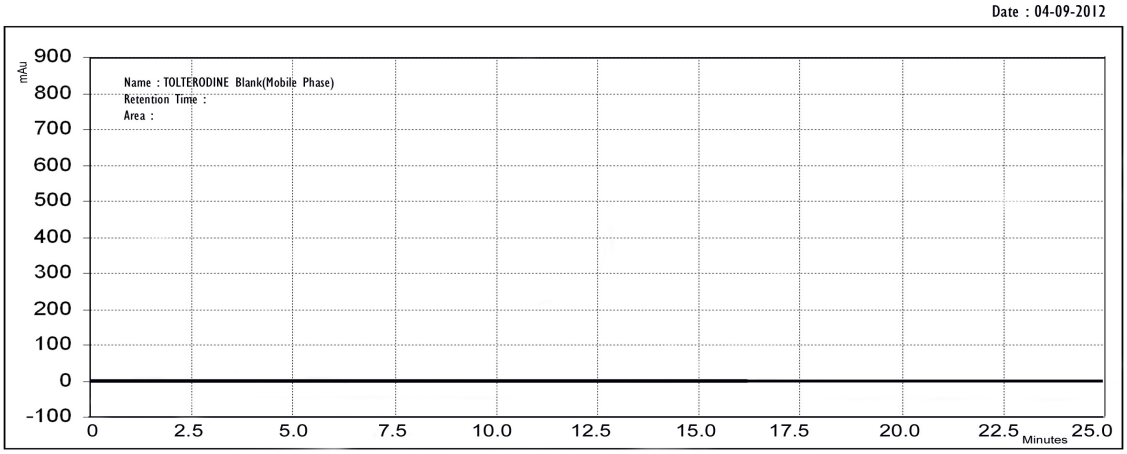


Fig 5: Standard

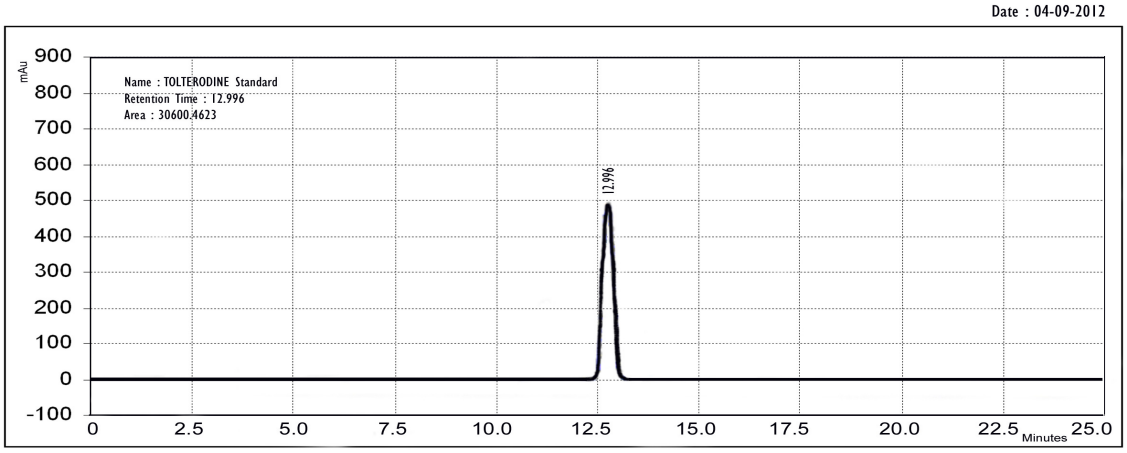


Fig 6: Sample

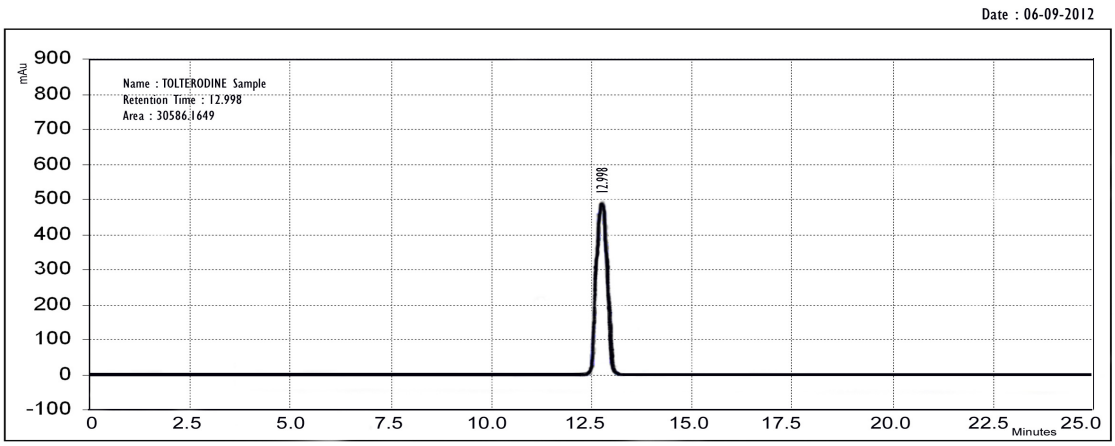


Fig 7: Linearity 1

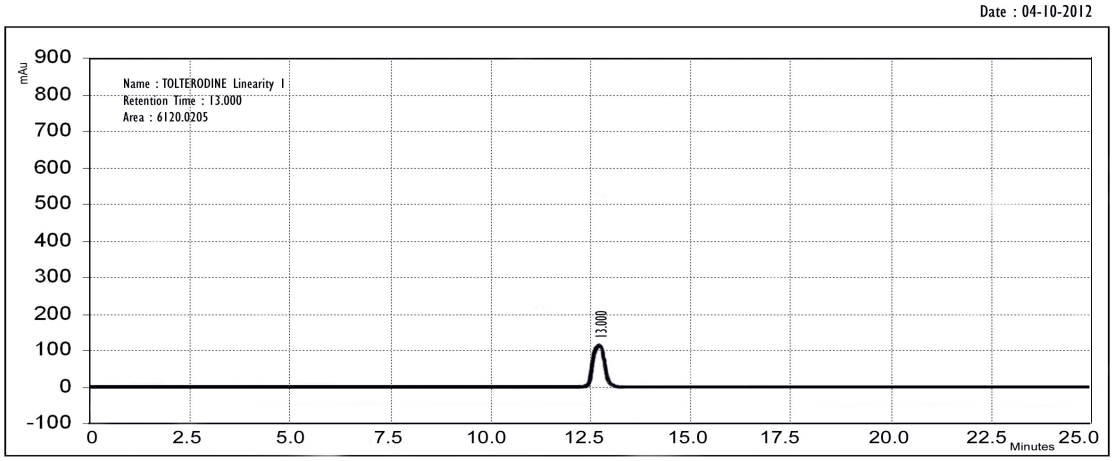


Fig 8: Linearity 2

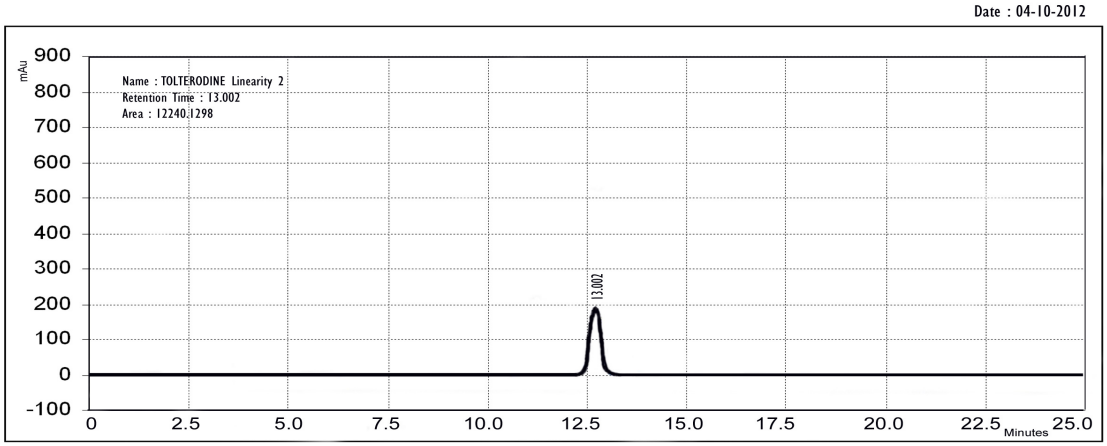


Fig 9: Linearity 3

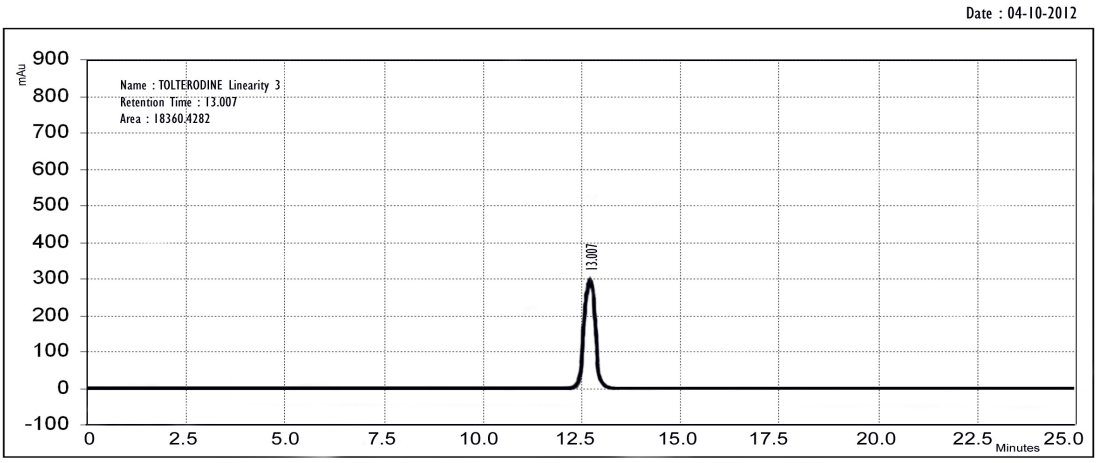


Fig 10 : Linearity 4

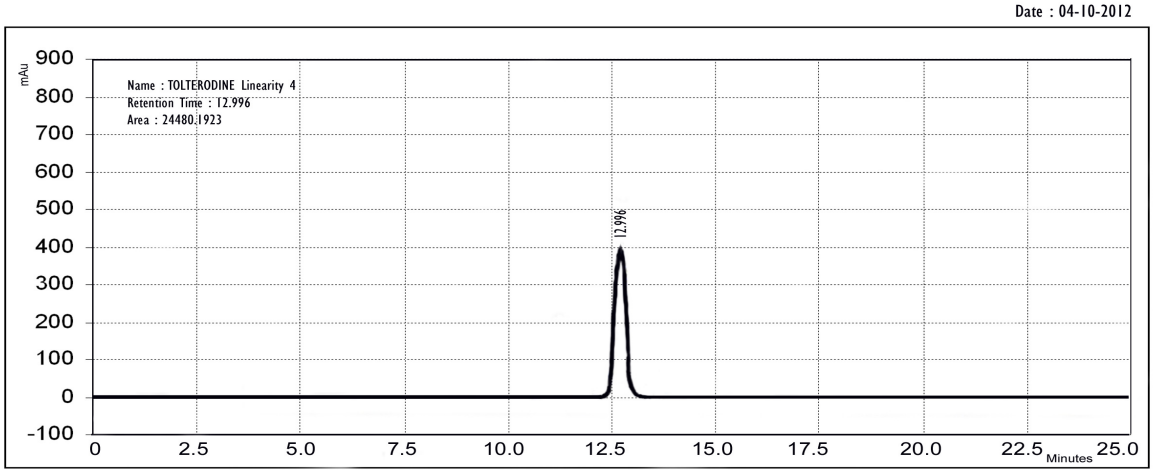


Fig 11: Linearity 5

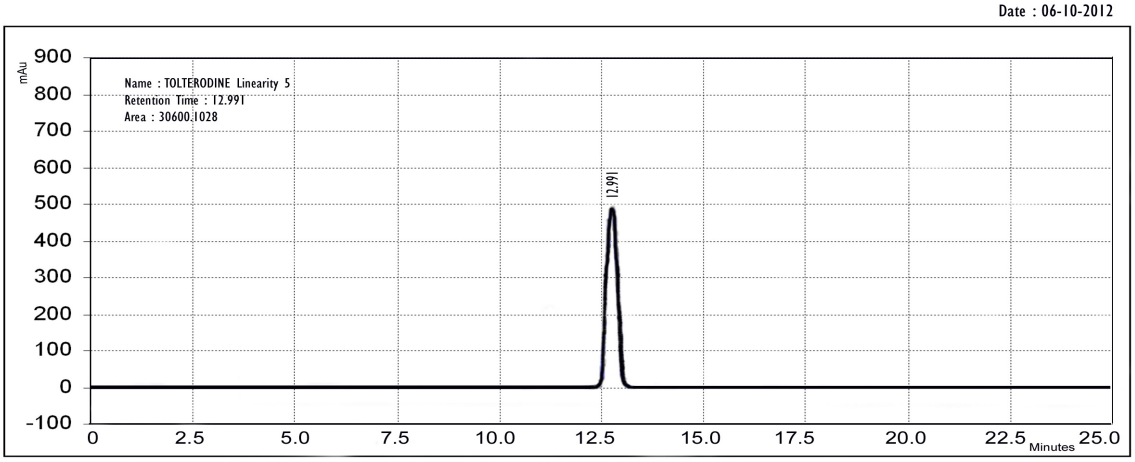


Fig 12: Linearity 6

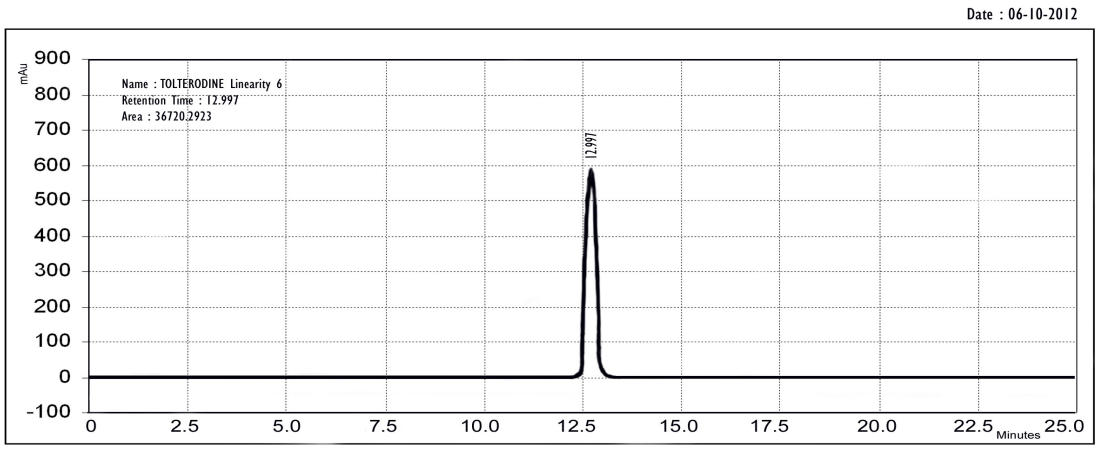


Fig 13: Linearity 7

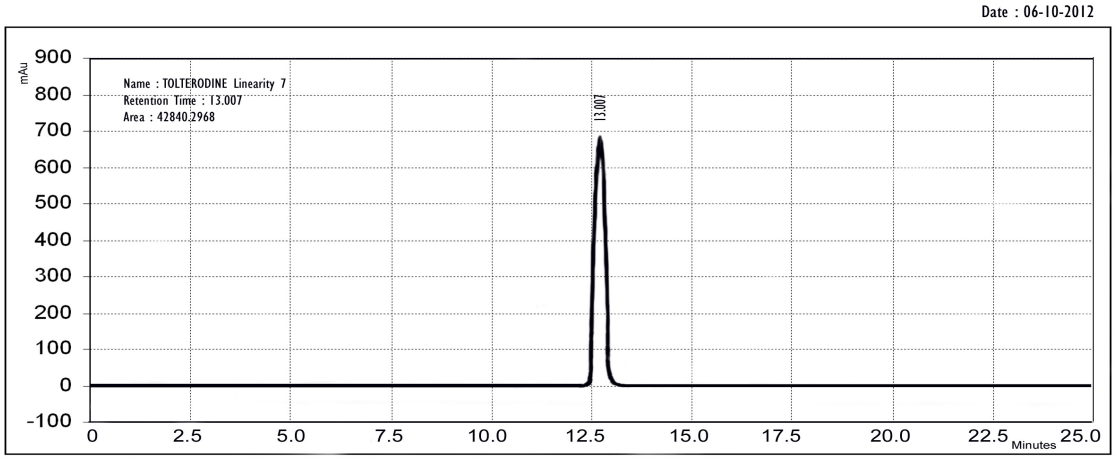


Fig 14: Linearity 8

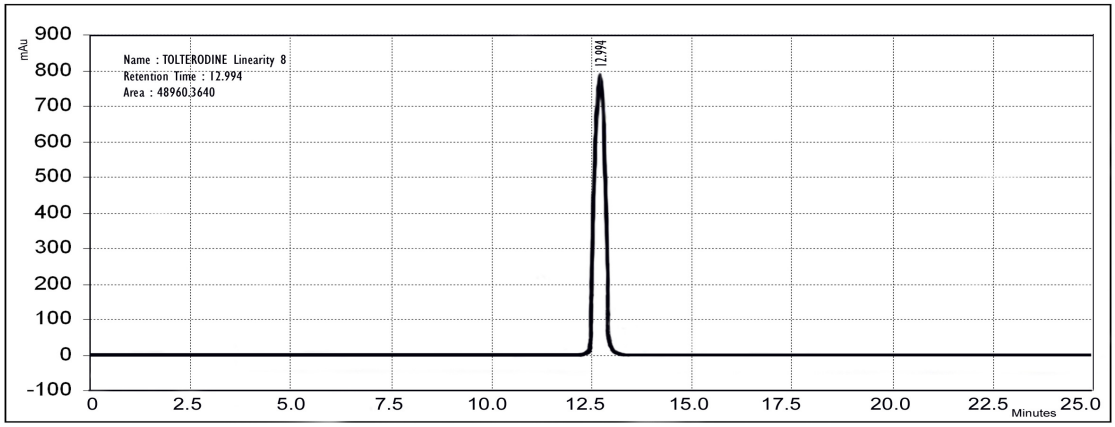


Fig 15: Linearity 9

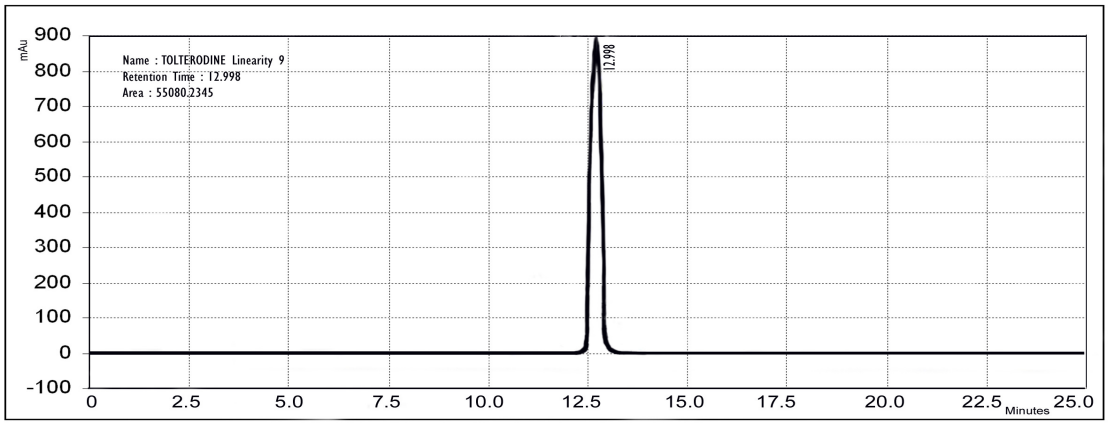


Fig 16: Linearity 10

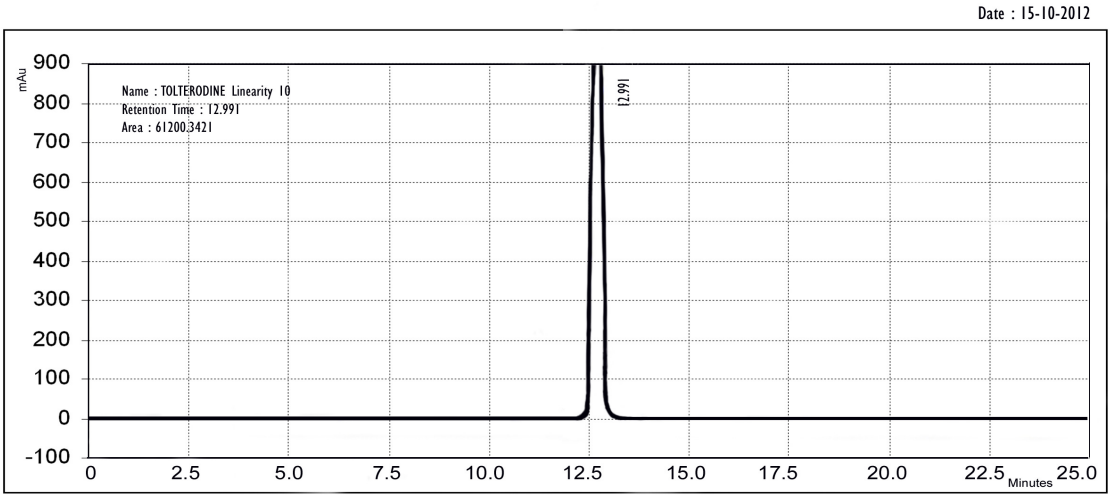


Fig 17: Linearity Calibration Curve

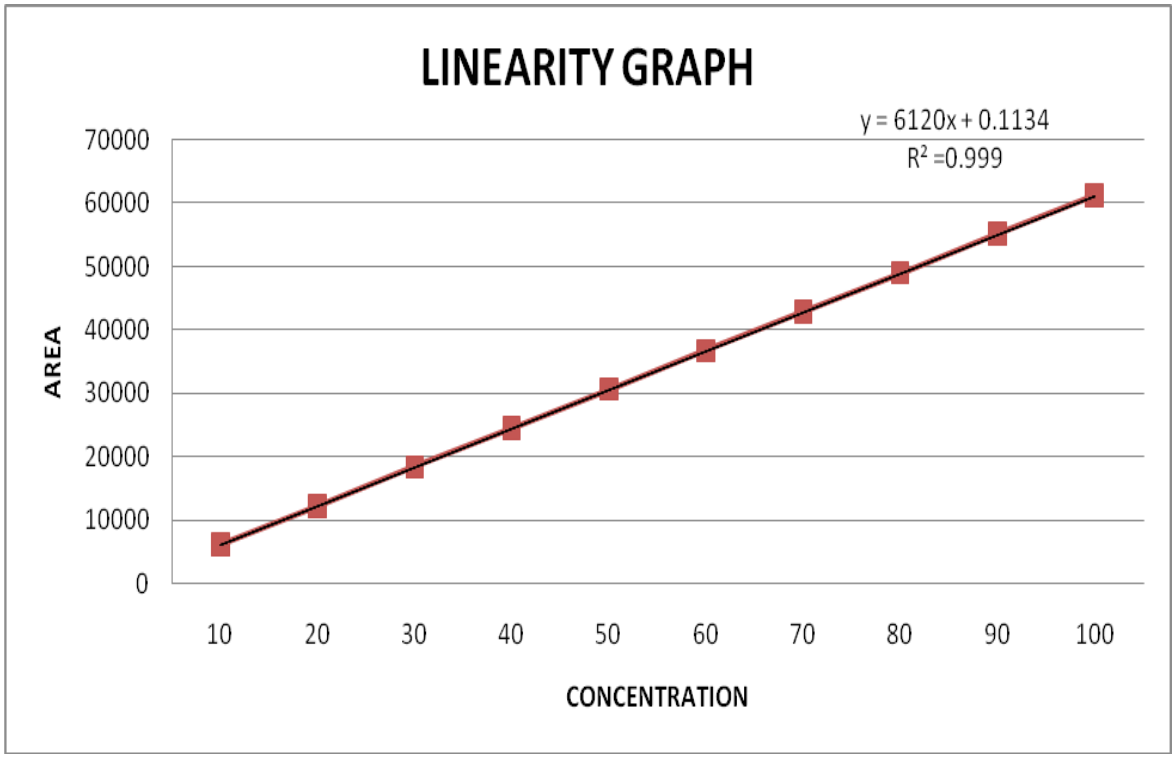


Fig 18: System precision 1

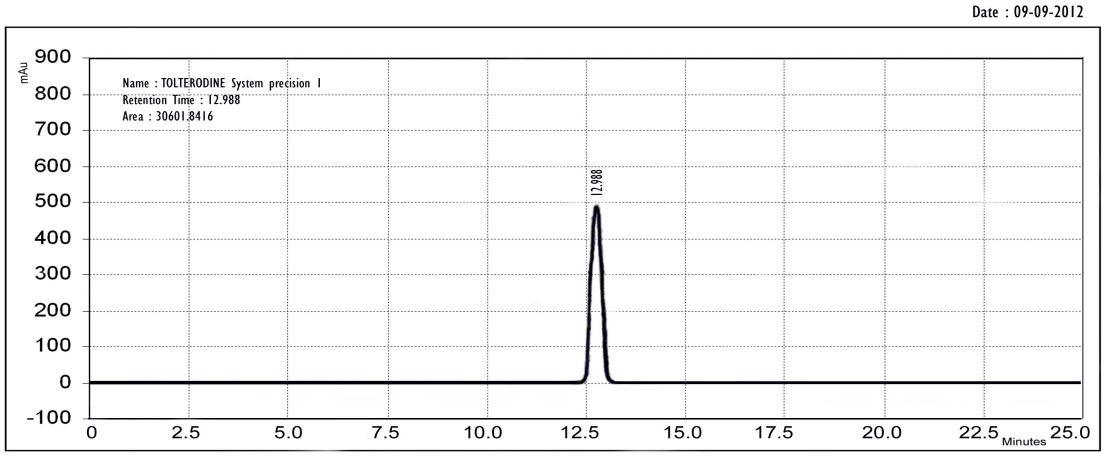


Fig 19: System precision 2

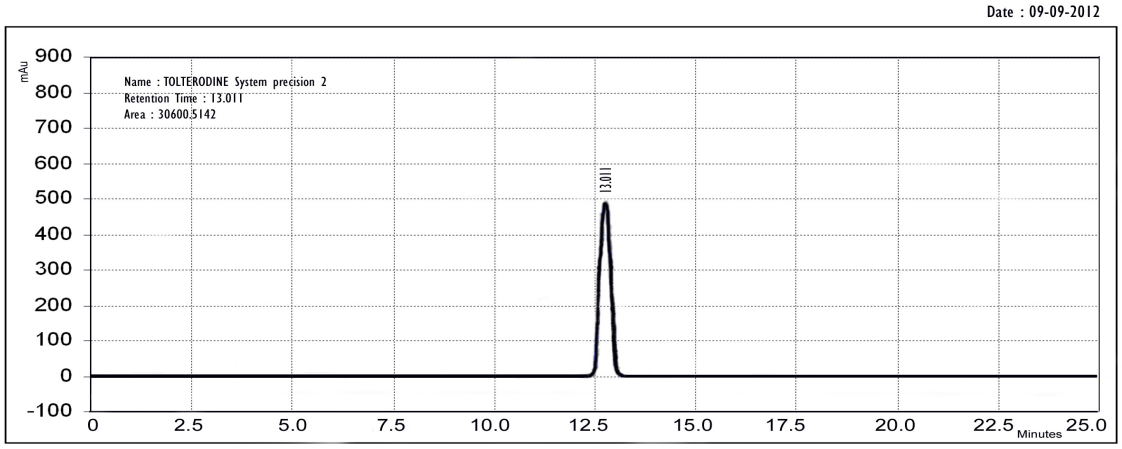


Fig 20: System precision 3

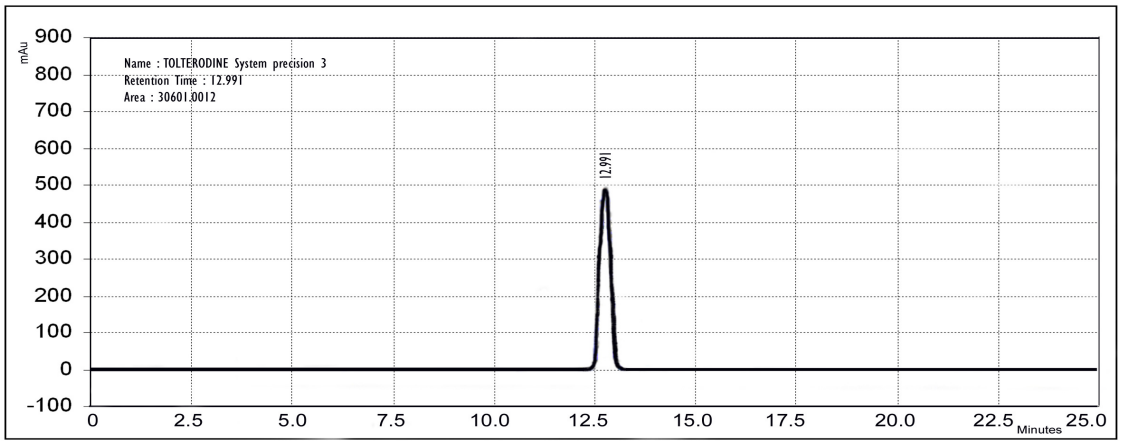


Fig 21: System precision 4

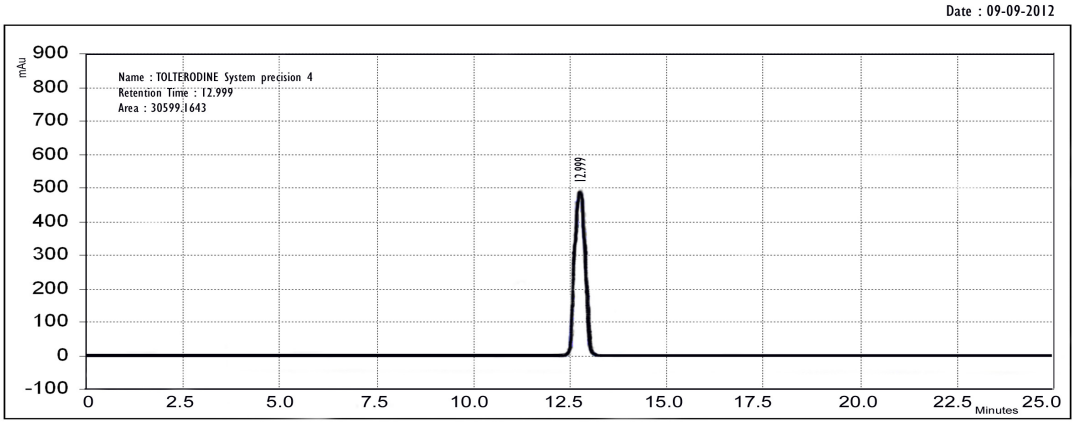


Fig 22: System precision 5

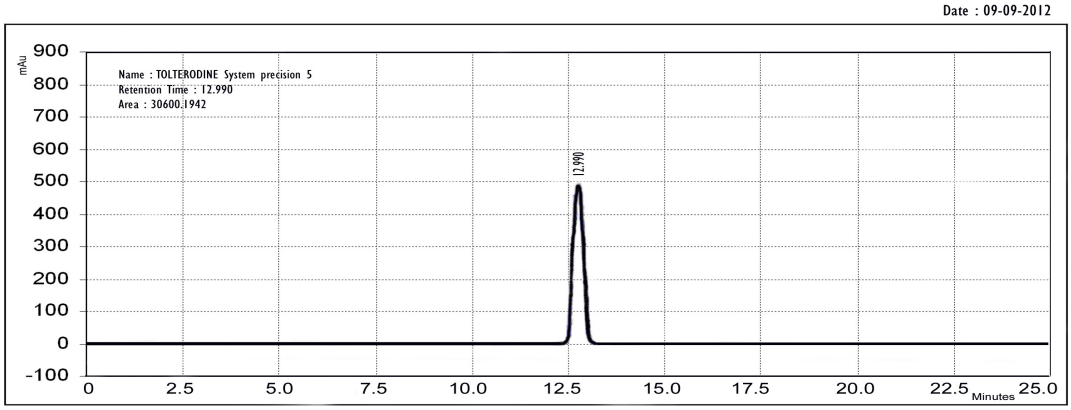


Fig 23: System precision 6

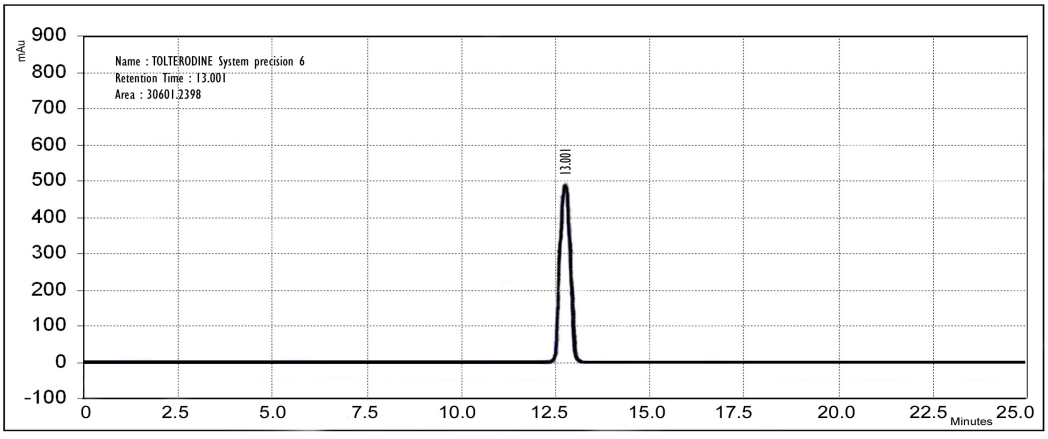


Fig 24: Method precision 1

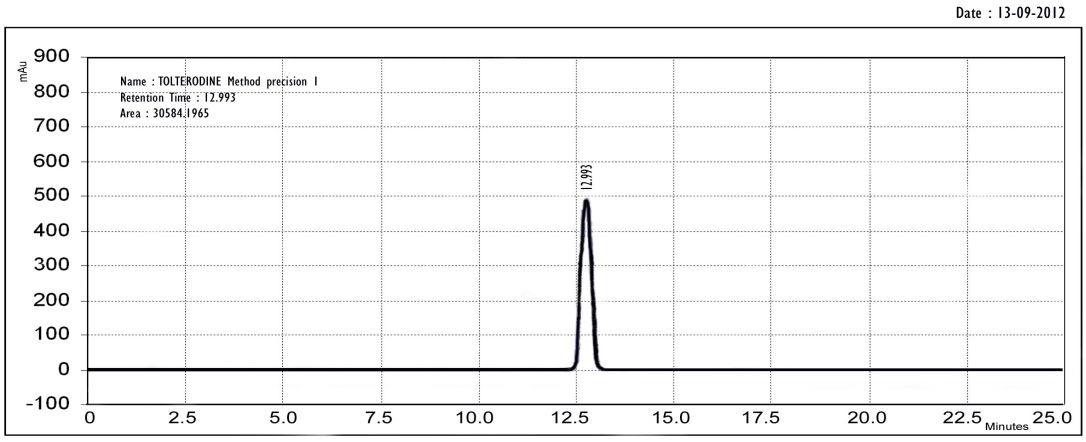


Fig 25: Method precision 2

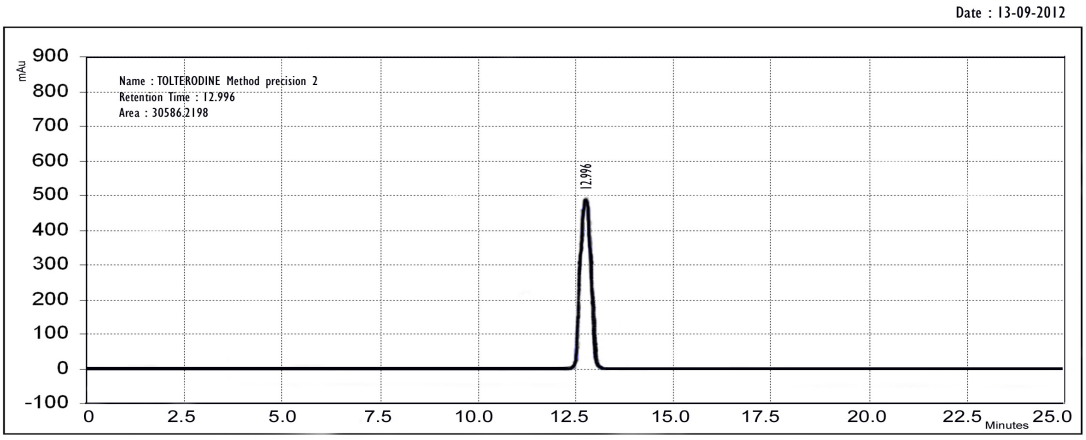


Fig 26: Method precision 3

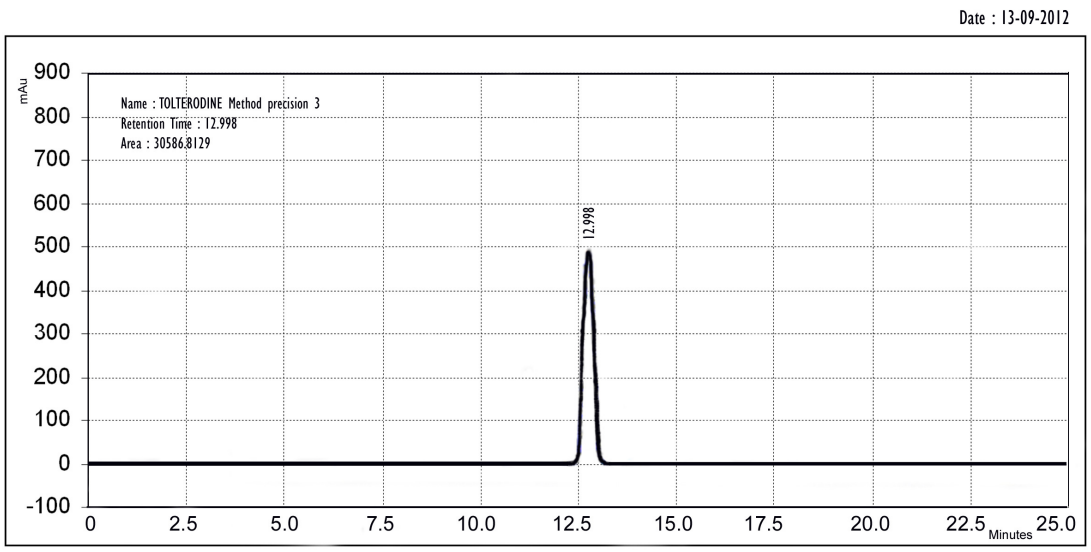


Fig 27: Method precision 4

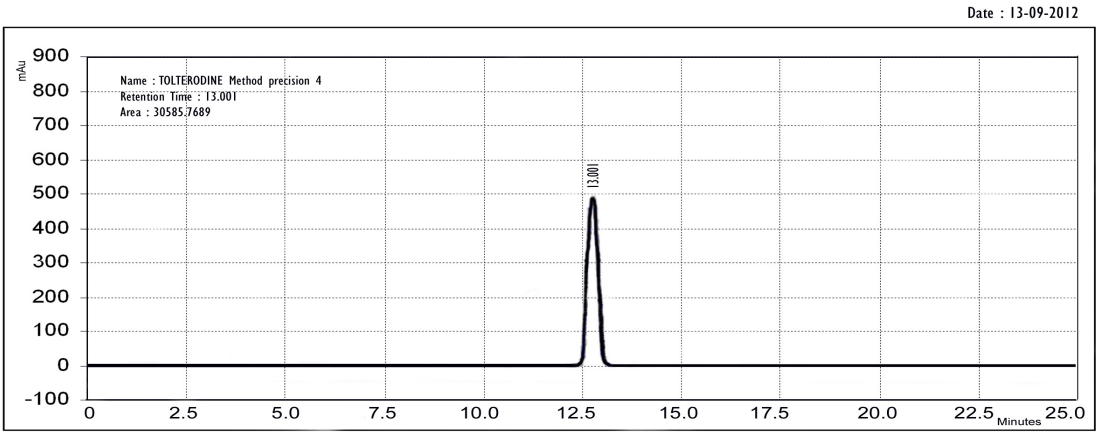


Fig 28: Method precision 5

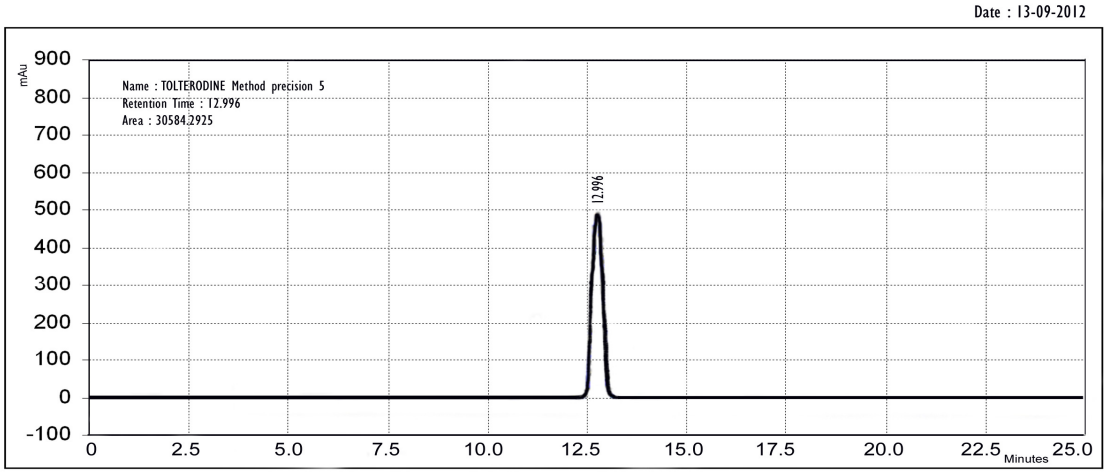


Fig 29: Method precision 6

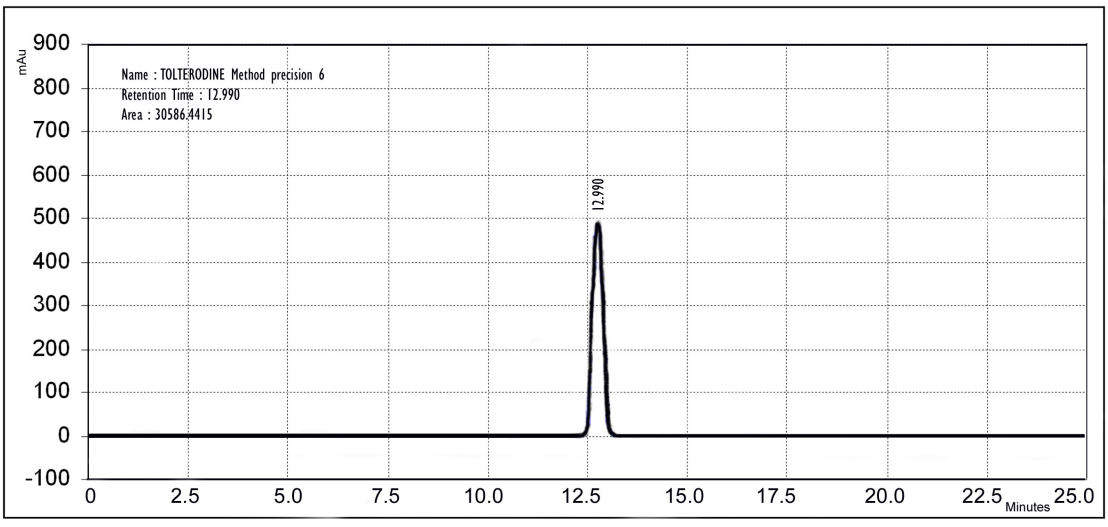


Fig 34: Accuracy 80% 1

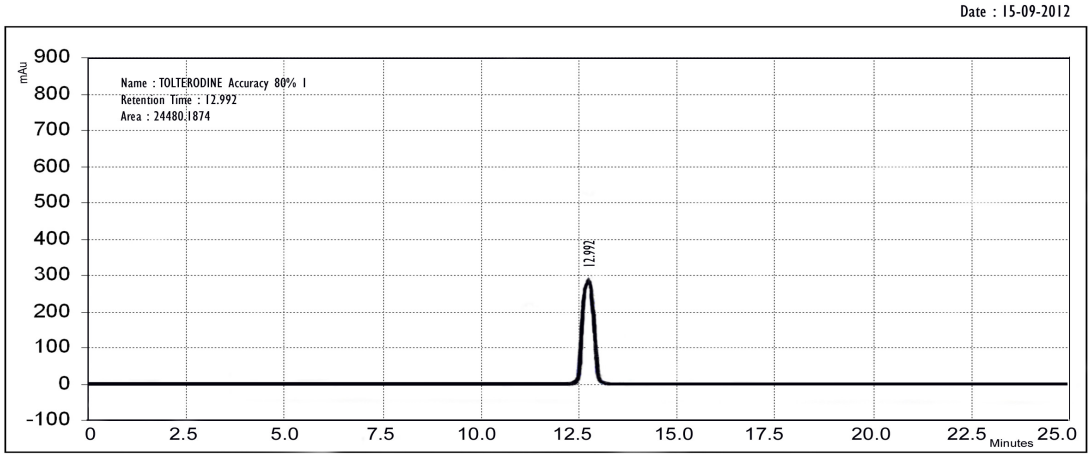


Fig 35: Accuracy 80% 2

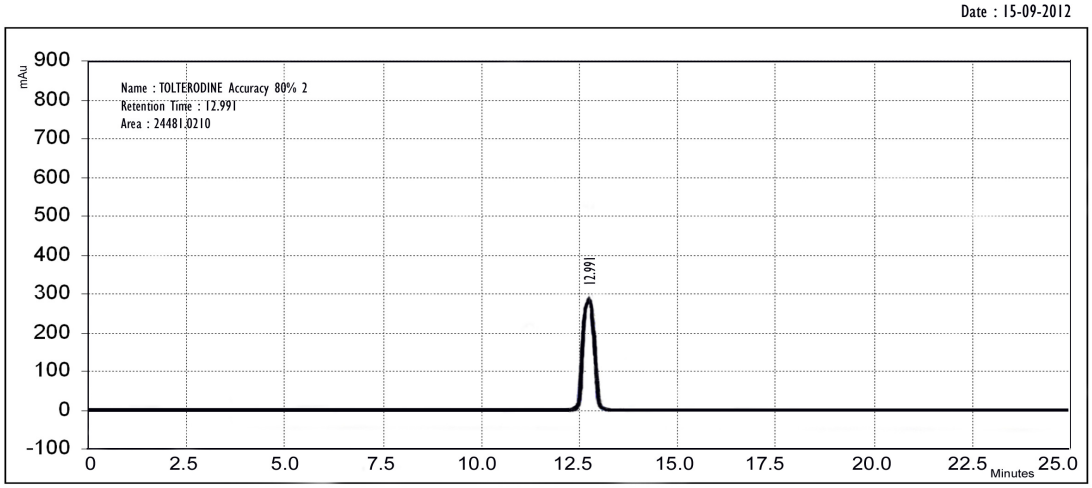


Fig 36: Accuracy 80% 3

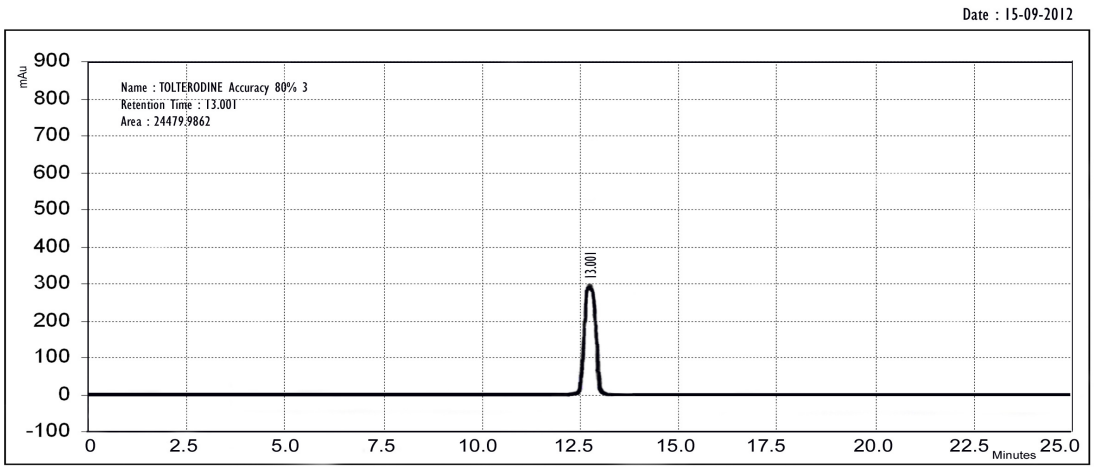


Fig 37: Accuracy 100% 1

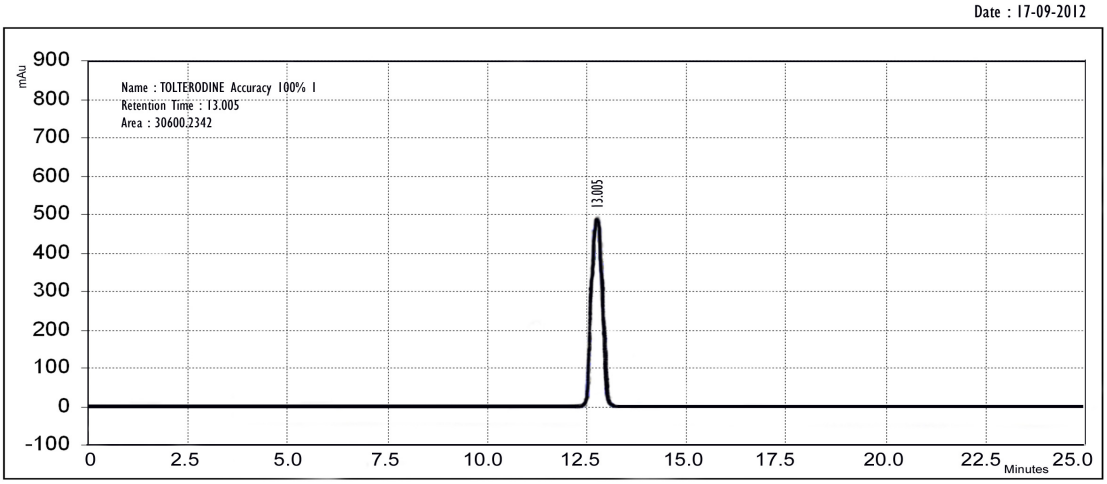


Fig 38: Accuracy 100% 2

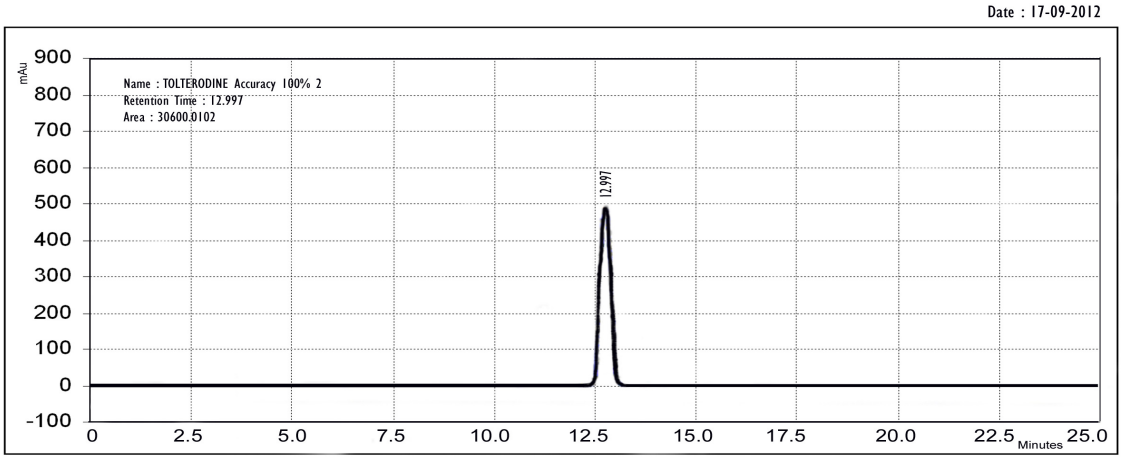


Fig 39: Accuracy 100% 3

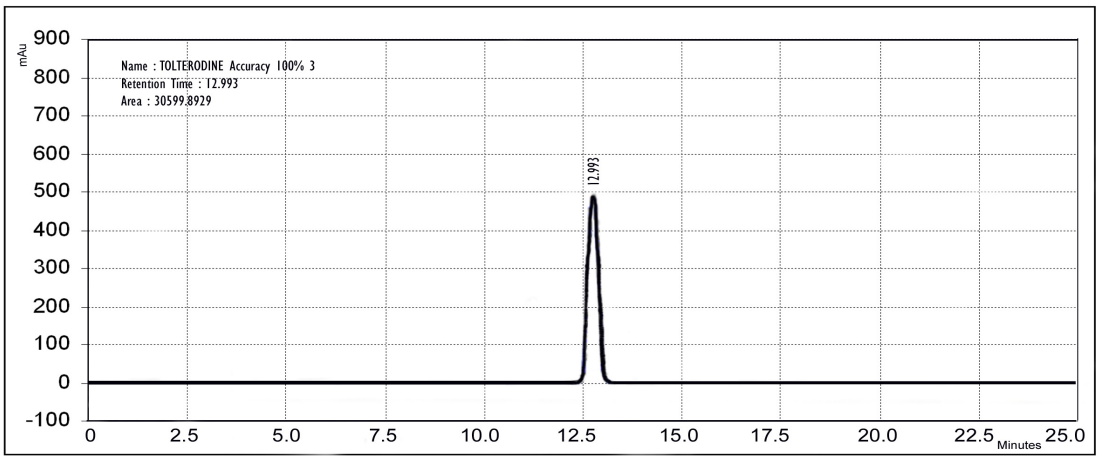


Fig 40: Accuracy 120% 1

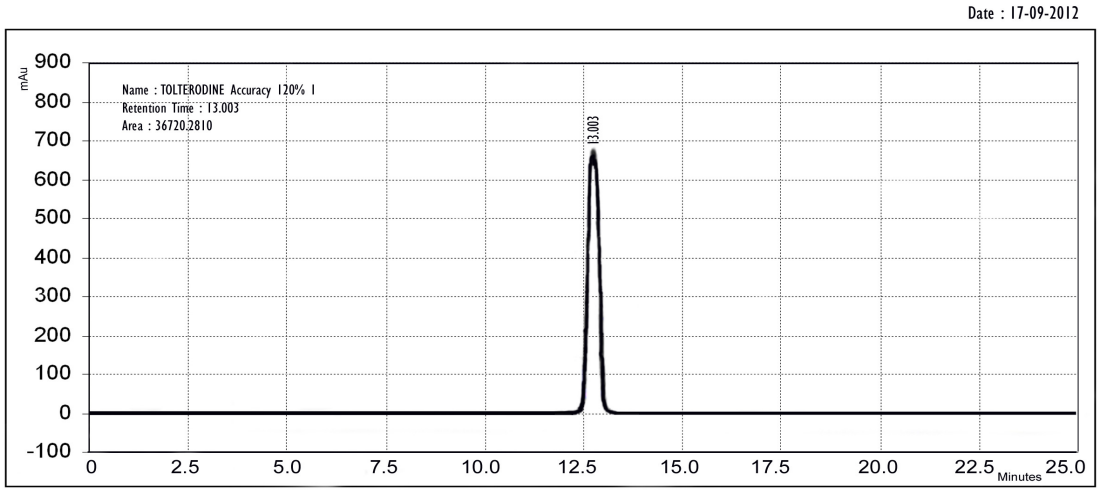


Fig 41: Accuracy 120% 2

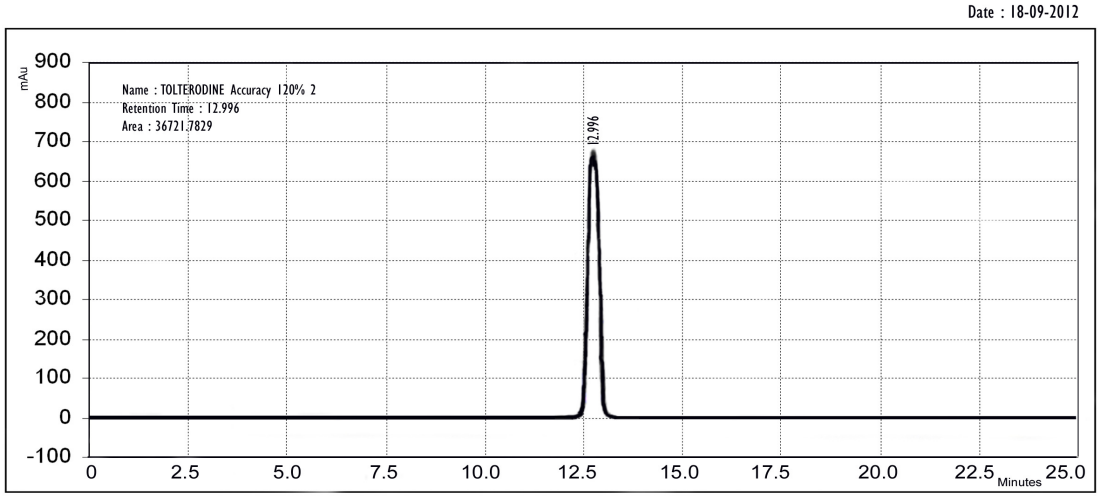


Fig 42: Accuracy 120% 3

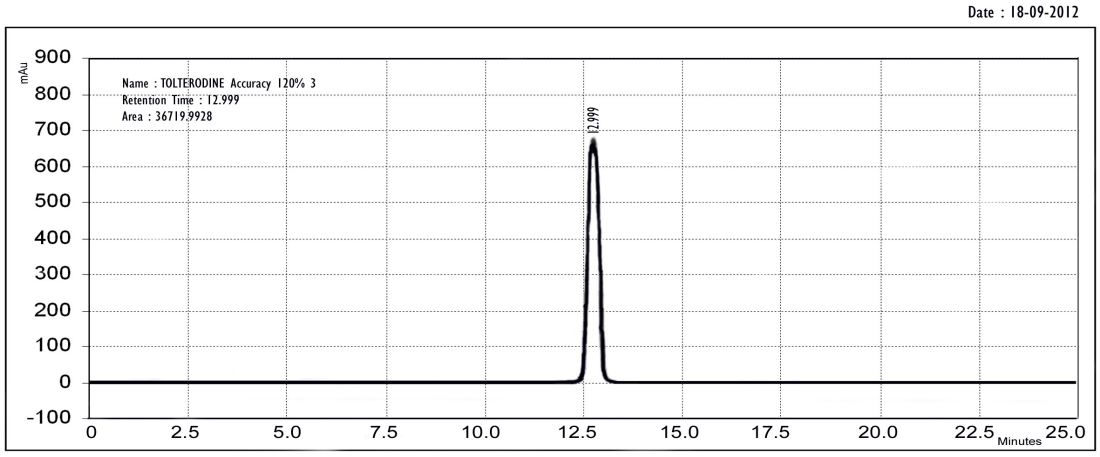


Fig 29: Blank

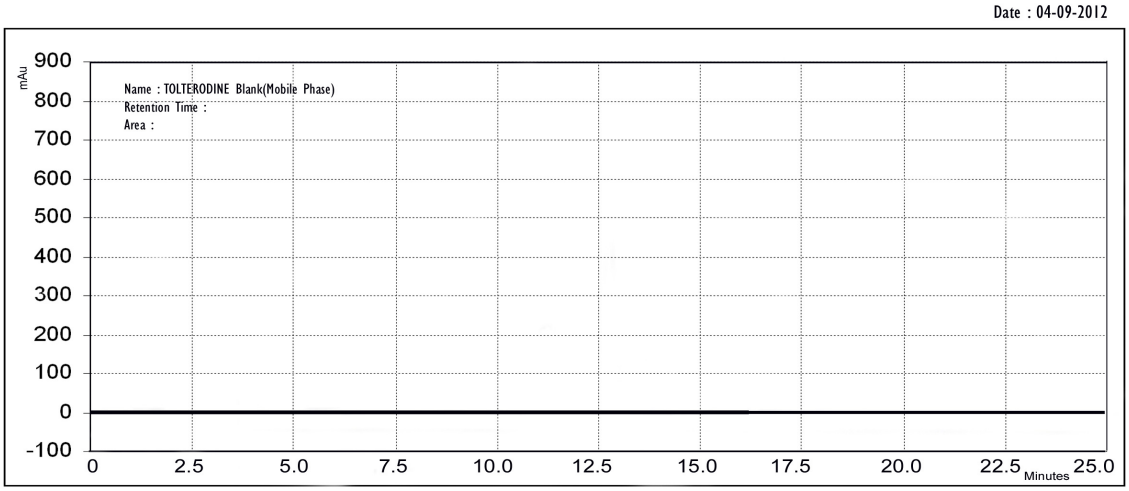


Fig 30: Placebo

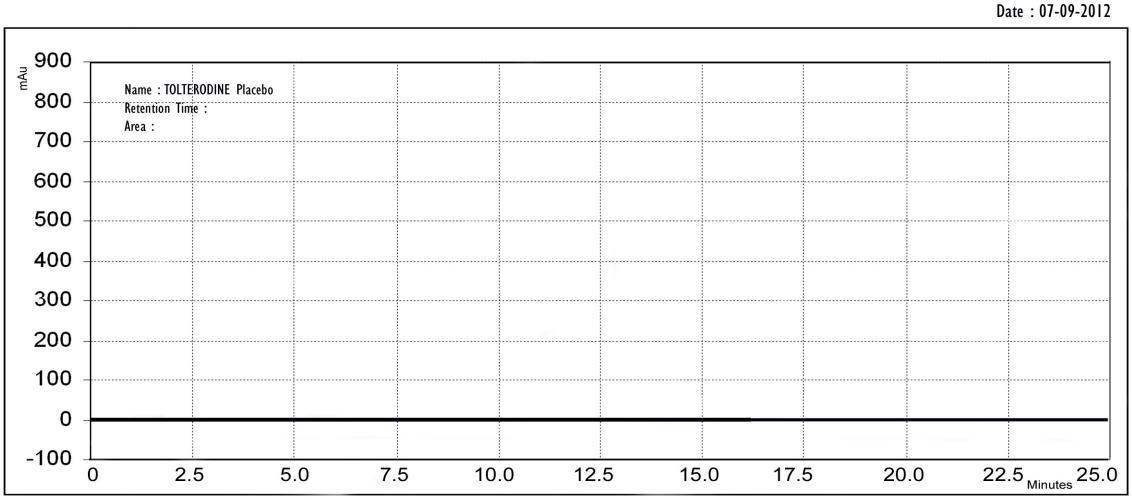


Fig 31: Standard

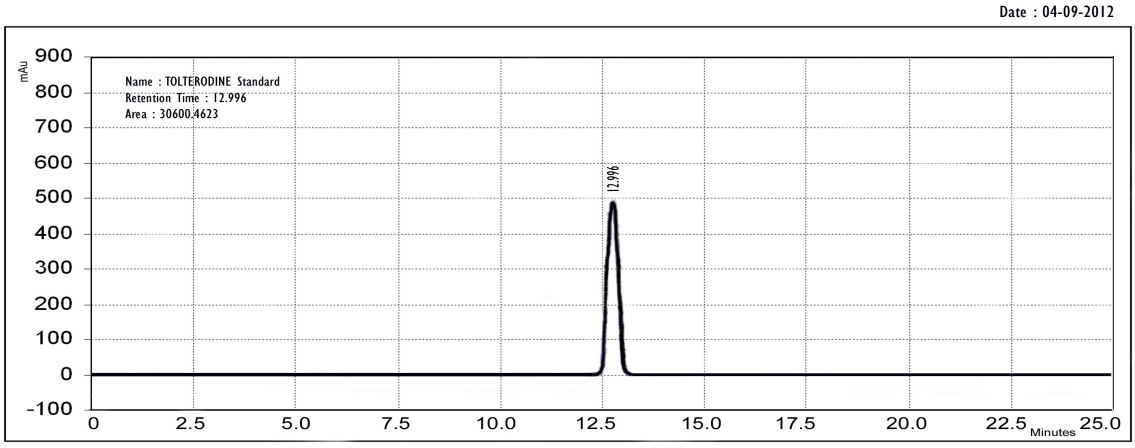


Fig 32: Standard + Placebo

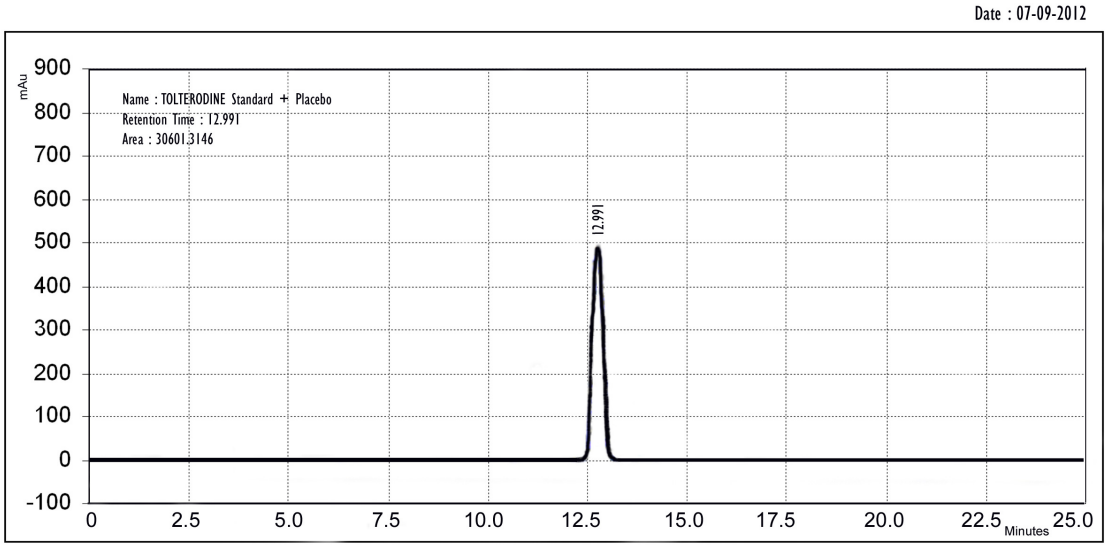


Fig 43: Ruggedness Standard I 1

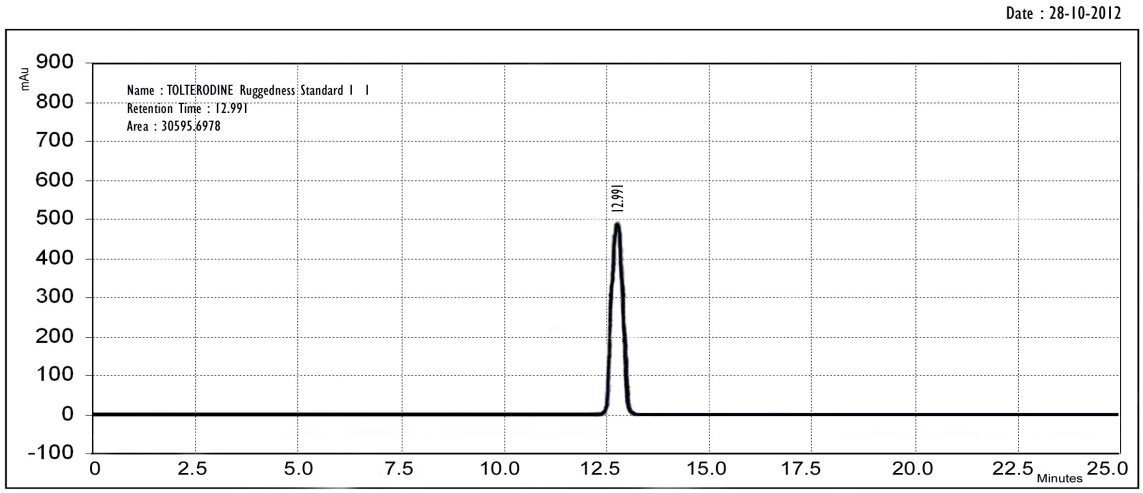


Fig 44:Ruggedness Sample I 1

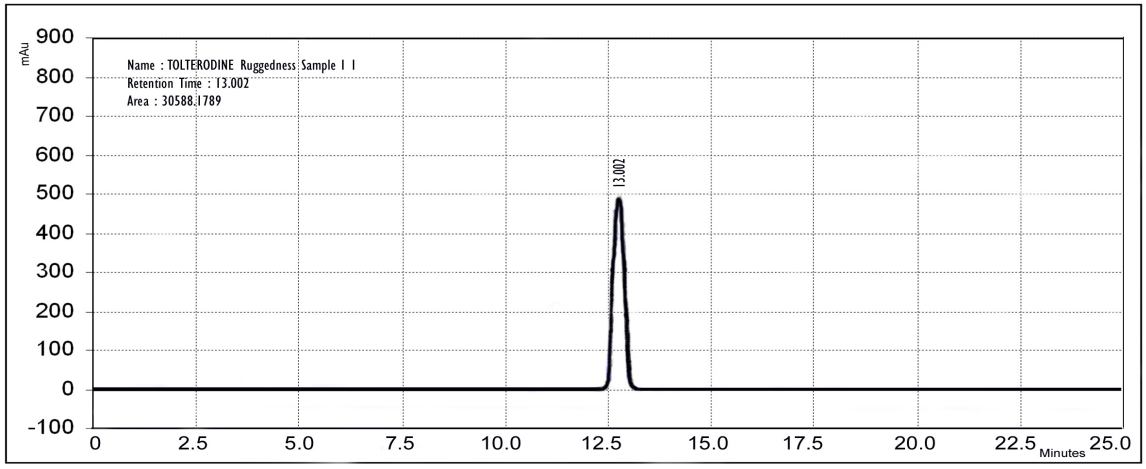


Fig 45: Ruggedness Standard I 2

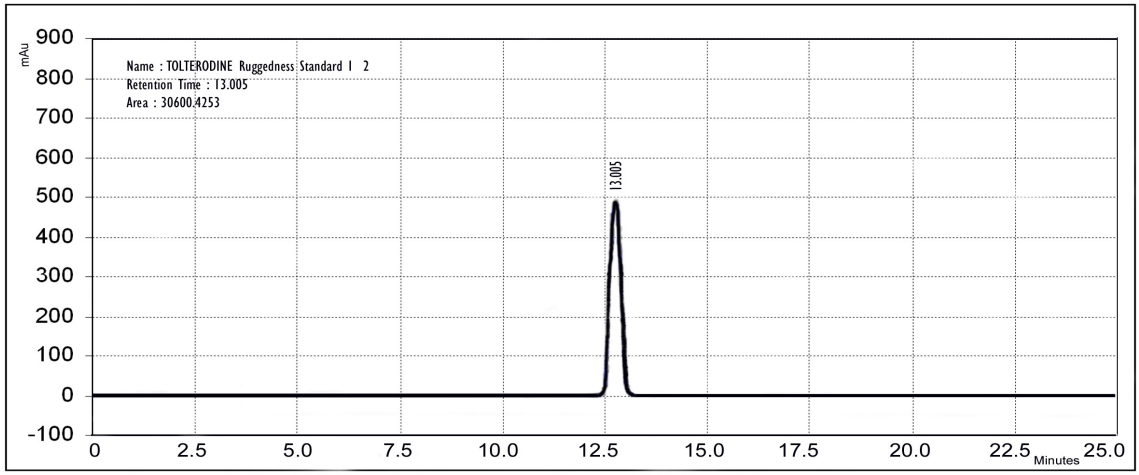


Fig 46: Ruggedness Sample I 2

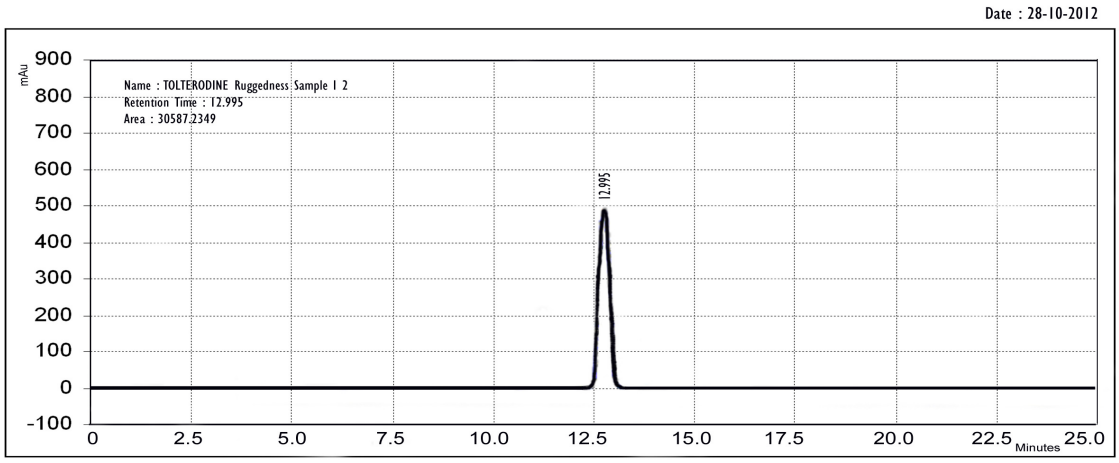


Fig 47: Ruggedness Standard II 1

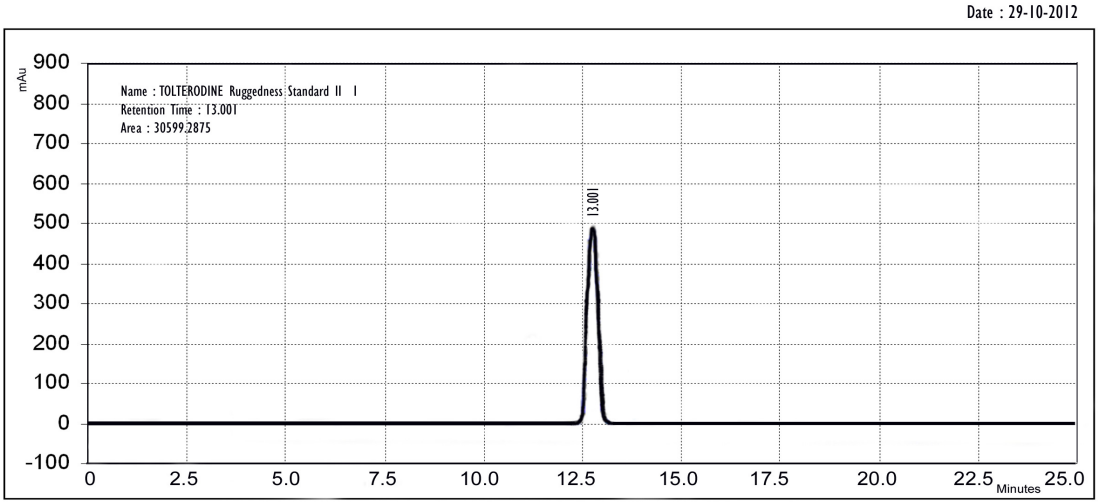


Fig 48: Ruggedness Sample II 1

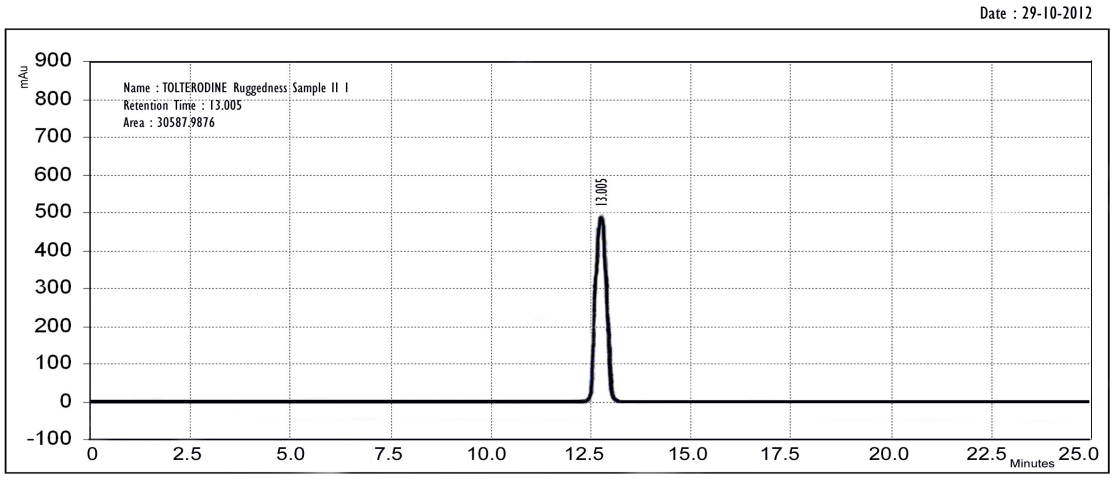


Fig 49: Ruggedness Standard II 2

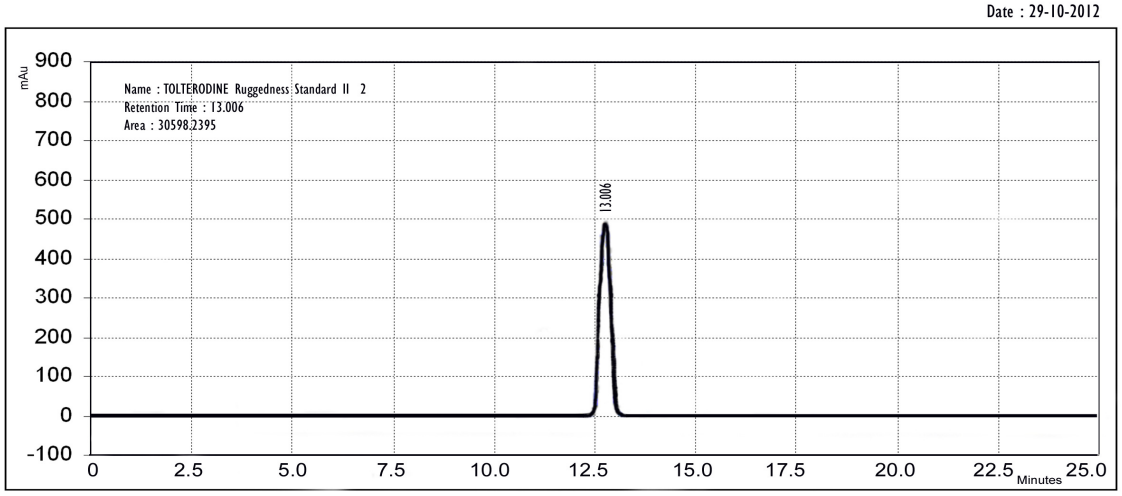


Fig 50: Ruggedness Sample II 2

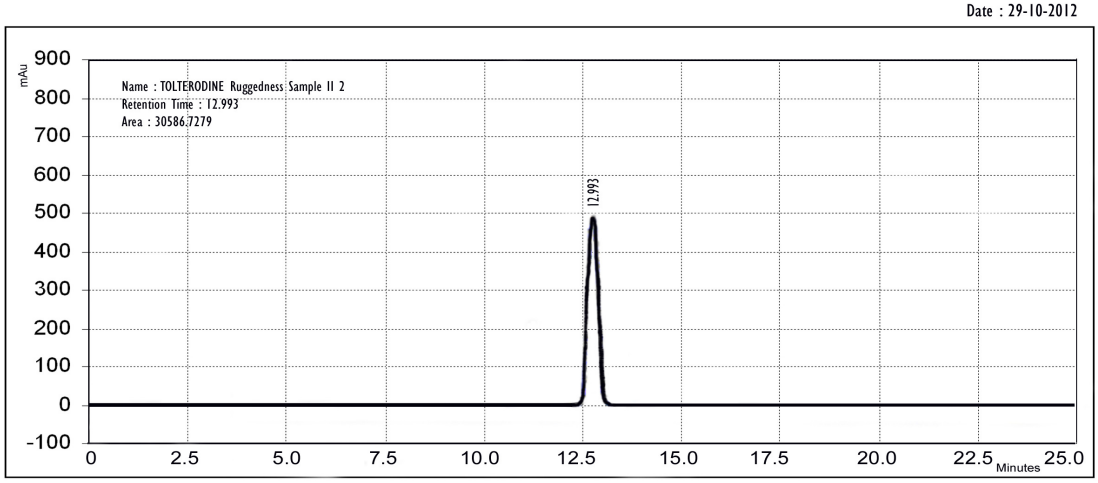


Fig 51: Ruggedness Standard I 1

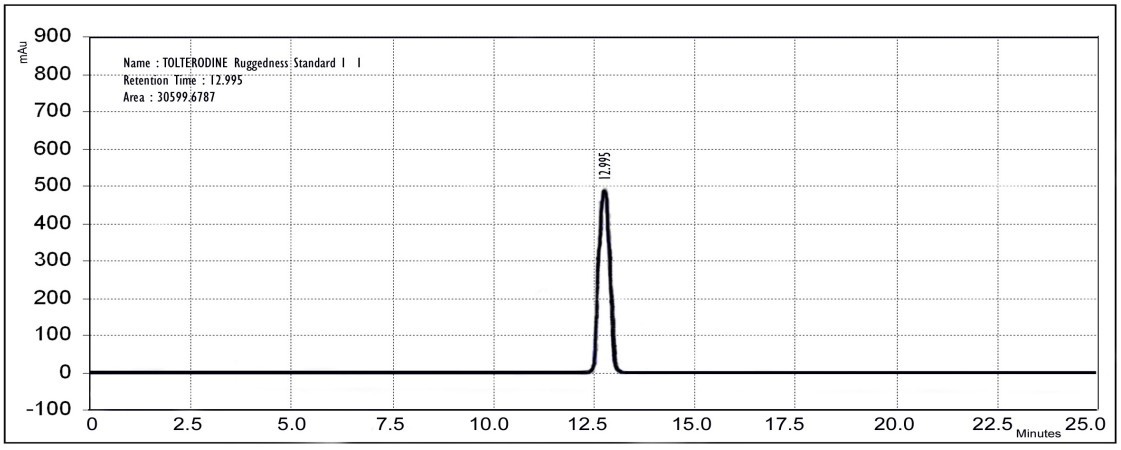


Fig 52: Ruggedness Sample I 1

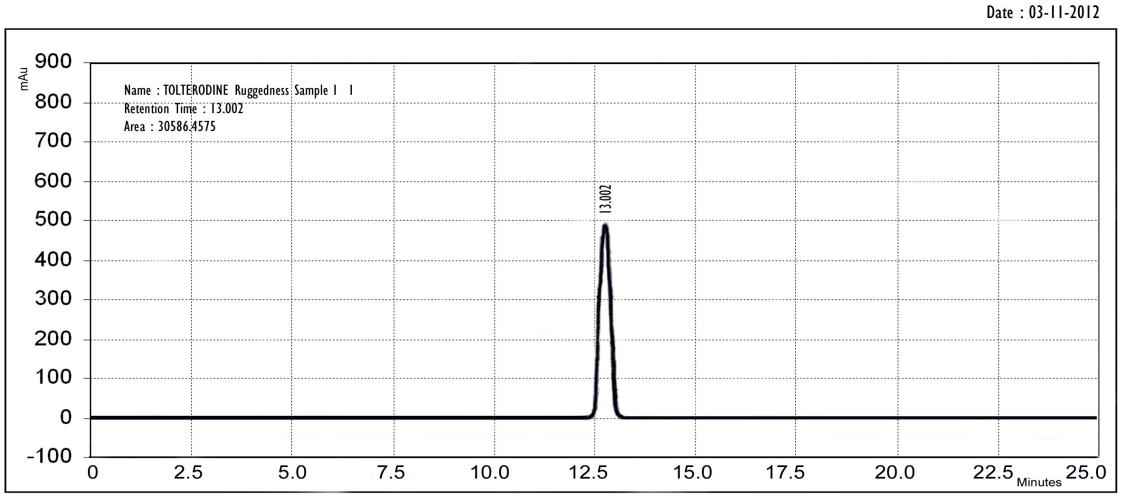


Fig 53: Ruggedness Standard I 2

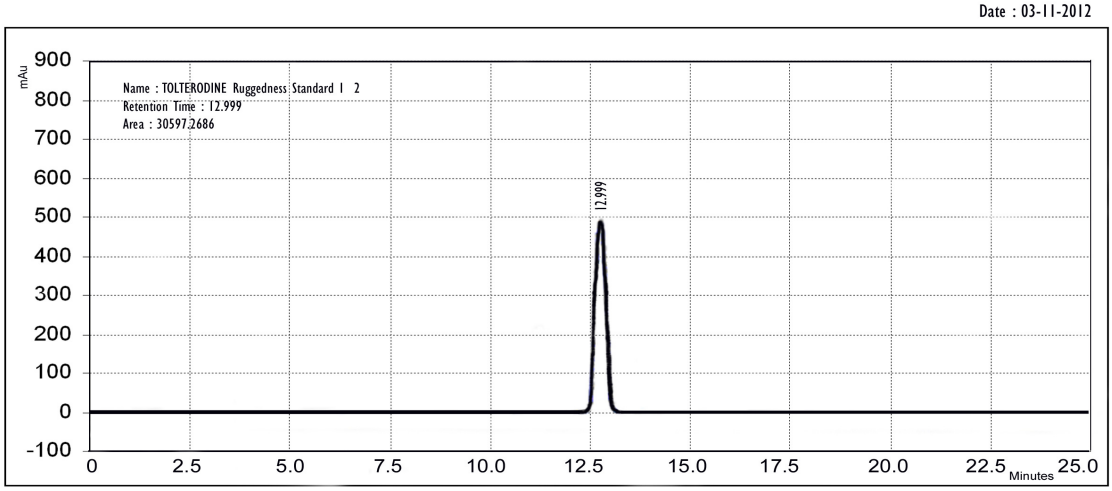


Fig 54: Ruggedness Sample I 2

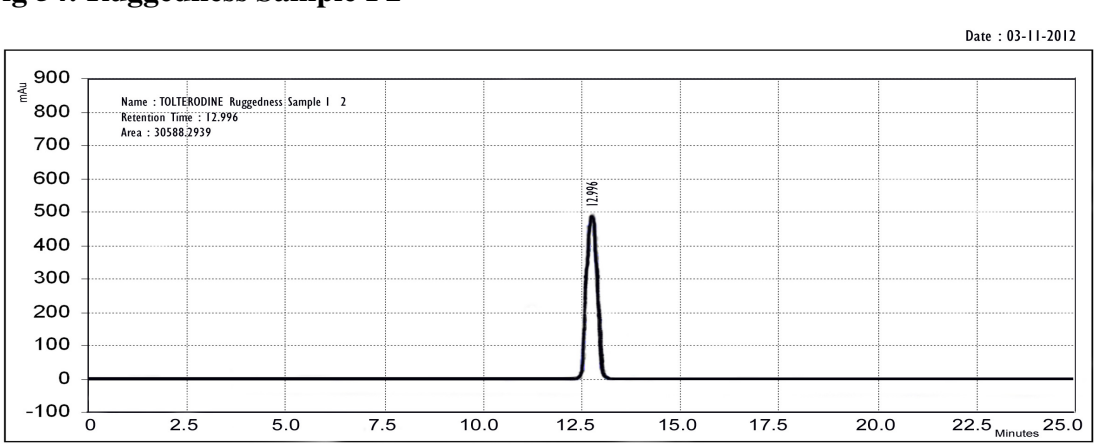


Fig 55: Ruggedness Standard I 3

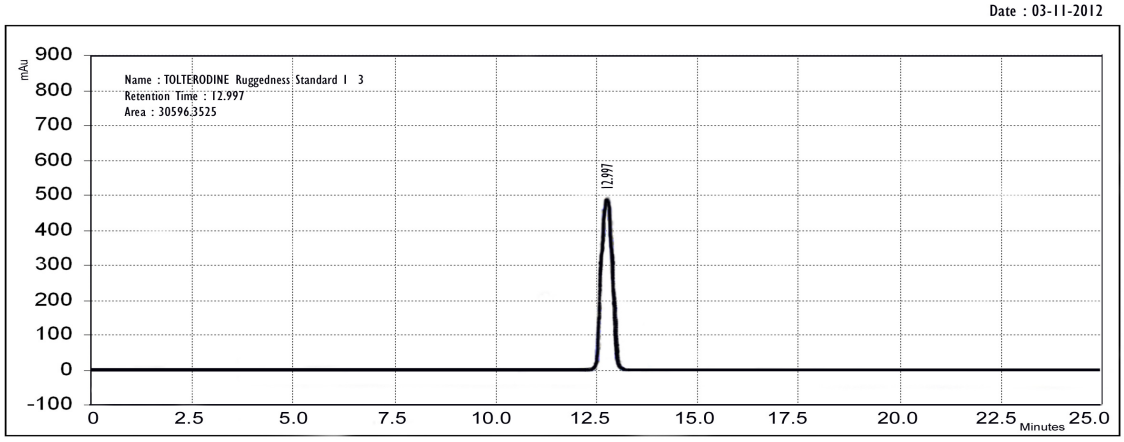


Fig 56: Ruggedness Sample I 3

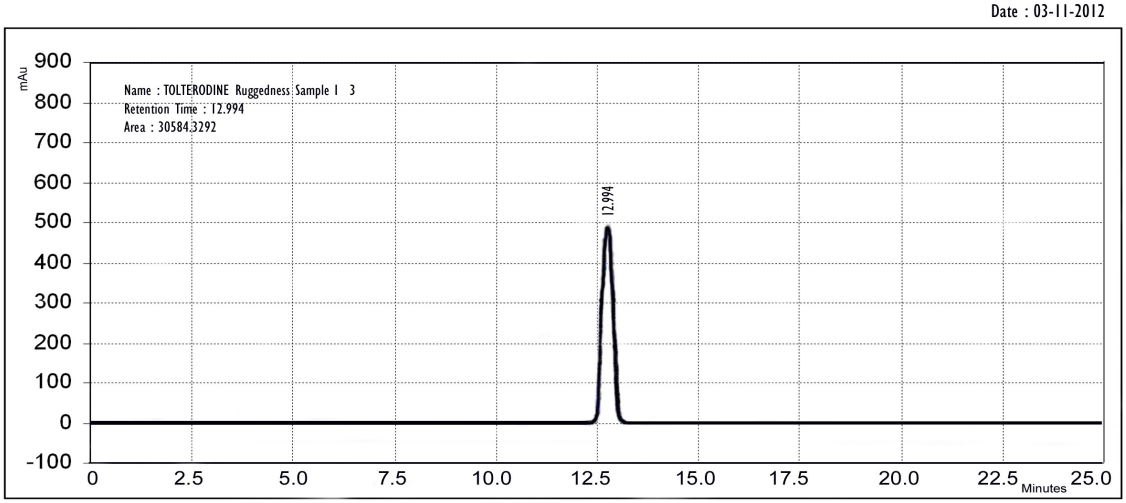


Fig 57: Ruggedness Standard I i 1

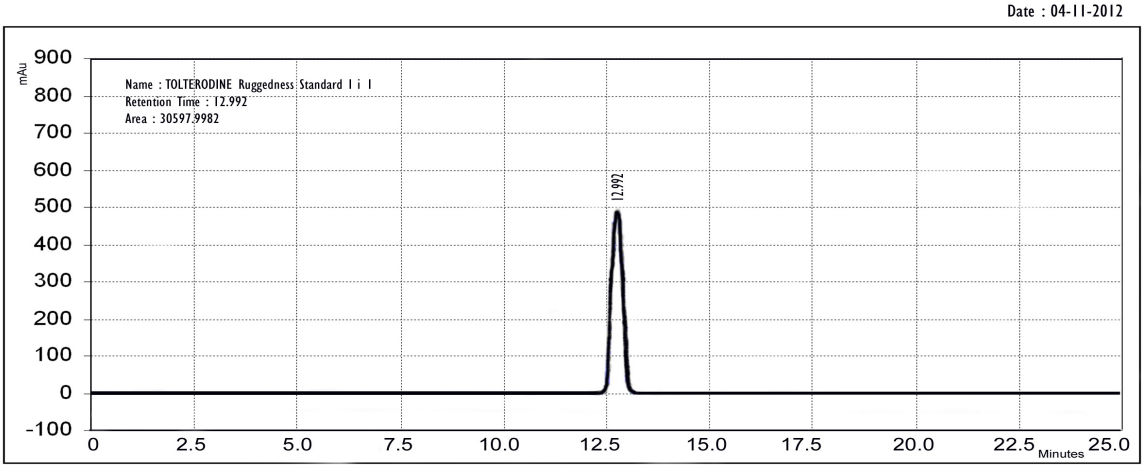


Fig 58: Ruggedness Sample I i 1

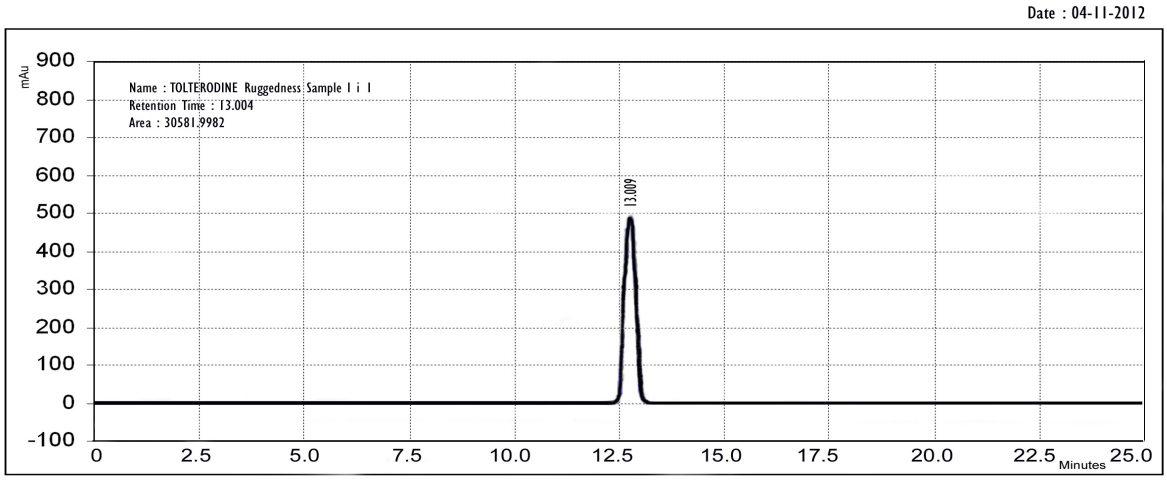


Fig 59: Ruggeddness Standard I i 2

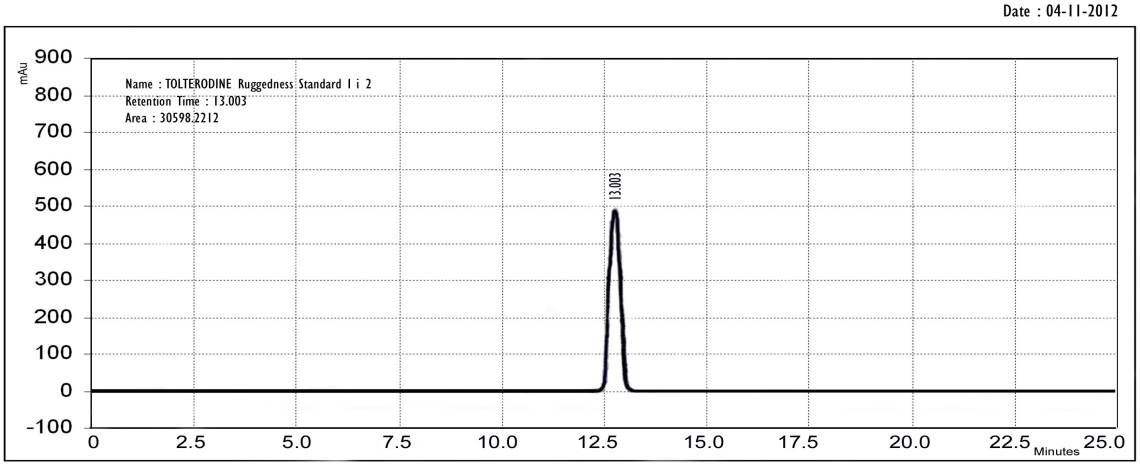


Fig 60: Ruggedness Sample I i 2

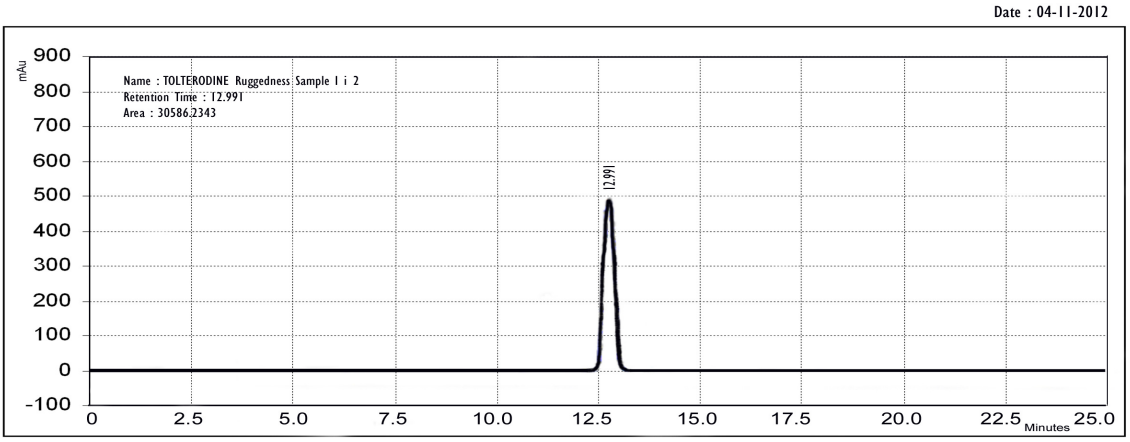


Fig 61: Ruggedness Standard I i 3

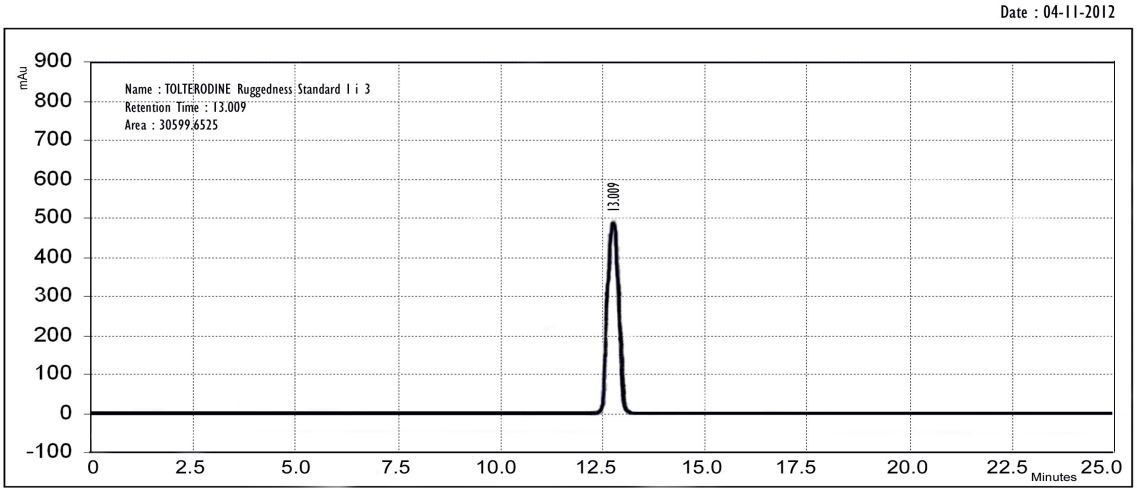


Fig 62: Ruggedness Sample I i 3

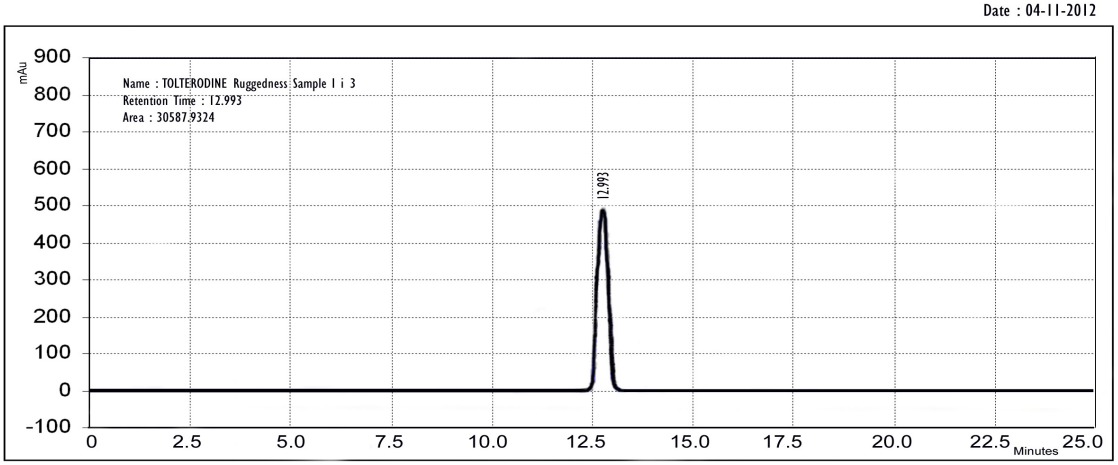


Fig 63: Robustness mobile phase(+) Standard 1(74:26)

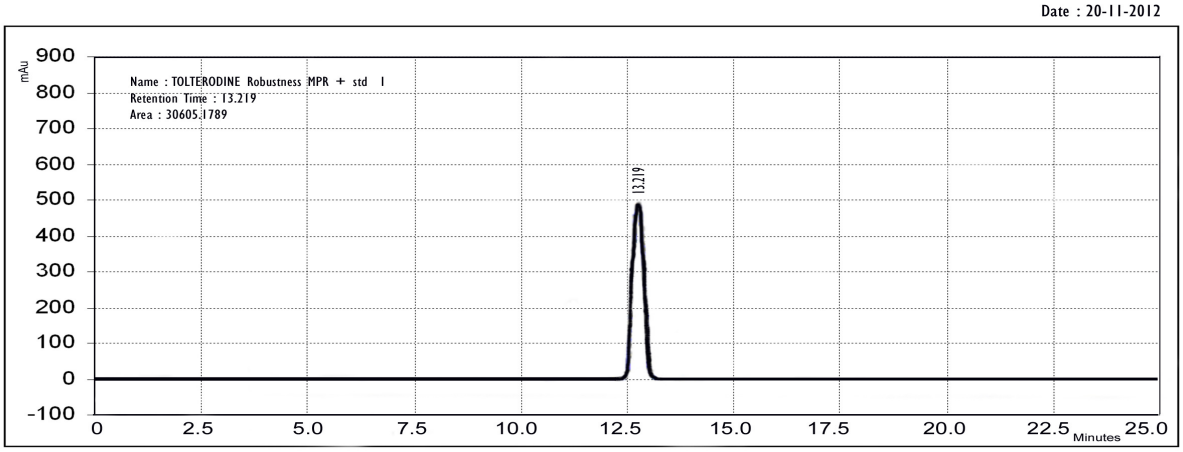


Fig 64: Robustness mobile phase(+) Standard 2(74:26)

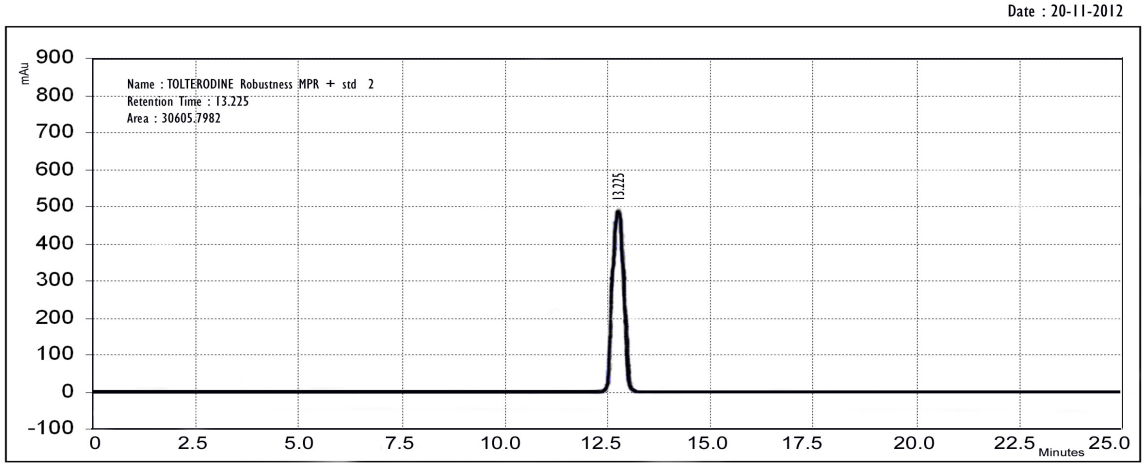


Fig 65: Robustness mobile phase(+) Standard 3(74:26)

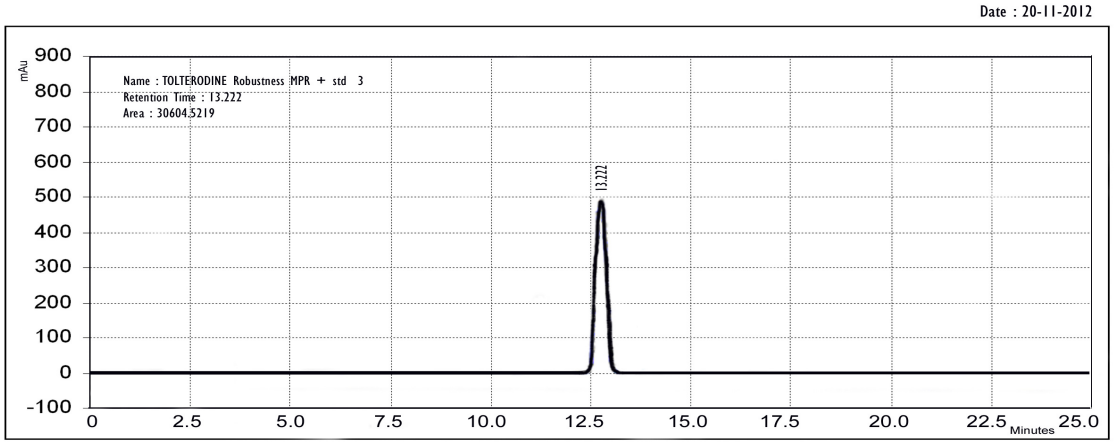


Fig 66: Robustness mobile phase(+) Sample 1(74:26)

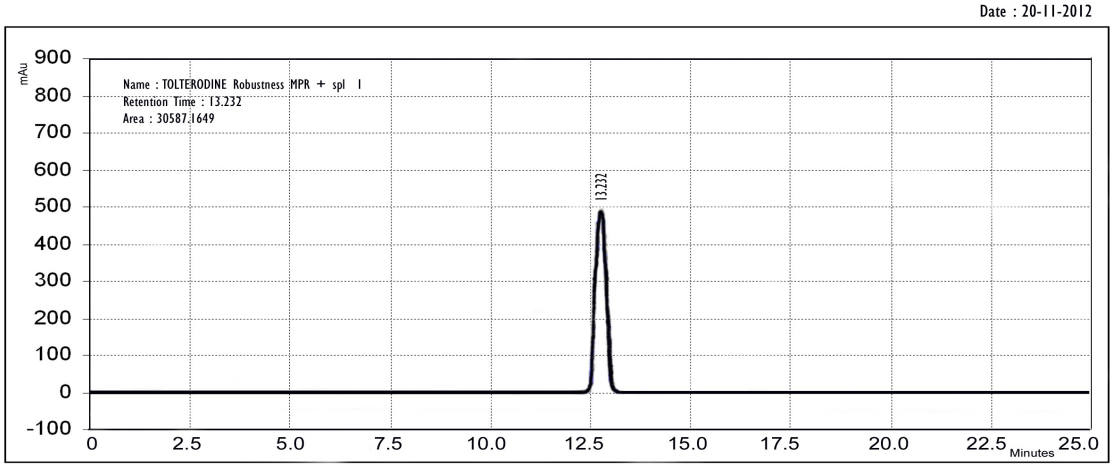


Fig 67: Robustness mobile phase(+) Sample 2(74:26)

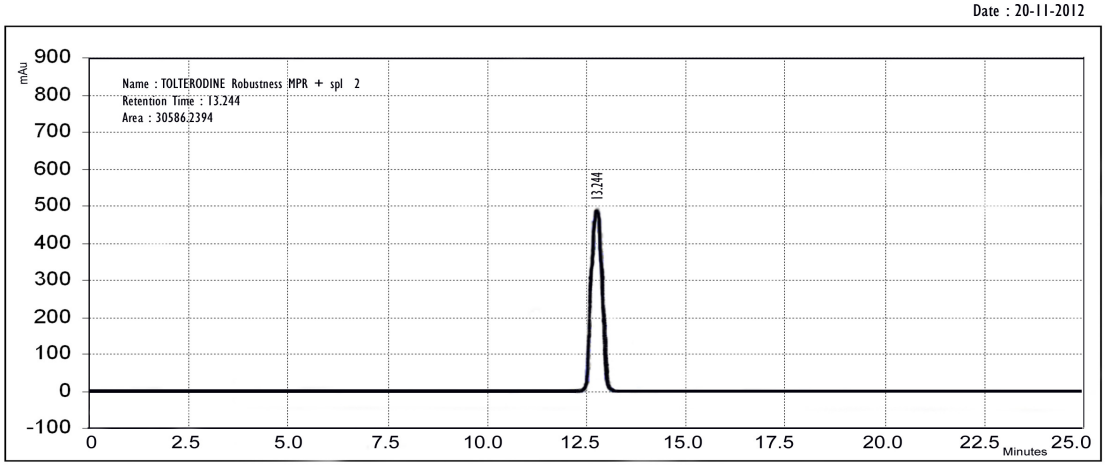


Fig 68: Robustness mobile phase(+) Sample 3(74:26)

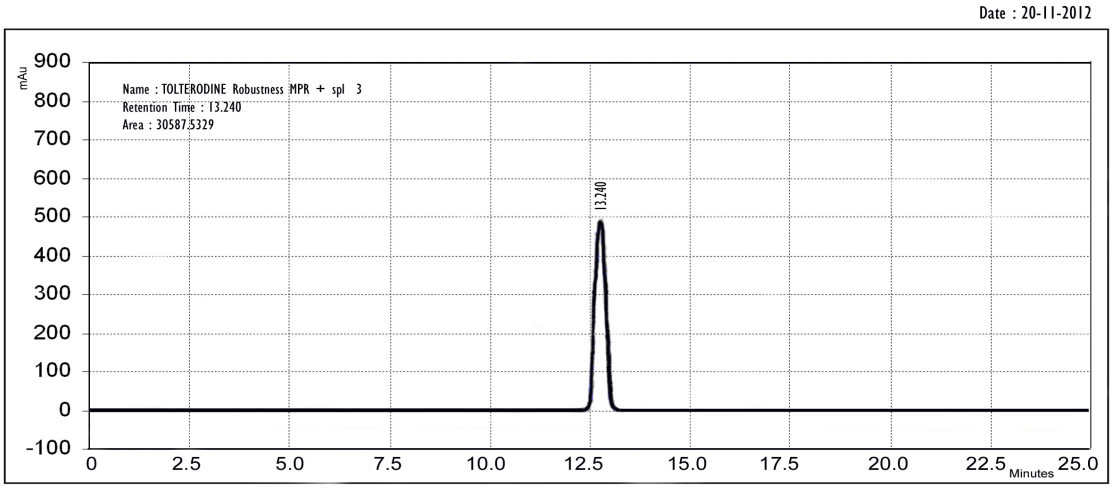


Fig 69: Robustness mobile phase(-) Standard 1(72:28)

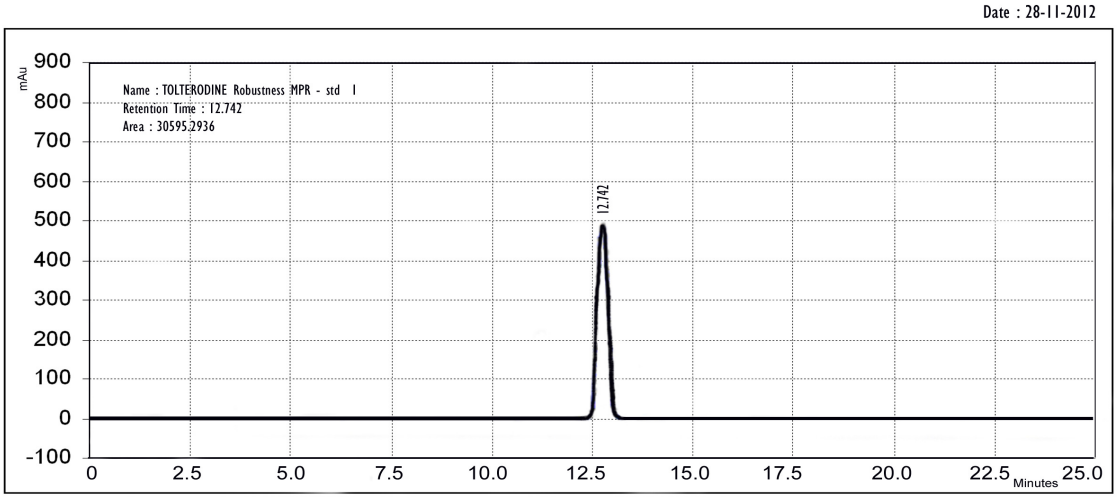


Fig 70: Robustness mobile phase(-) Standard 2(72:28)

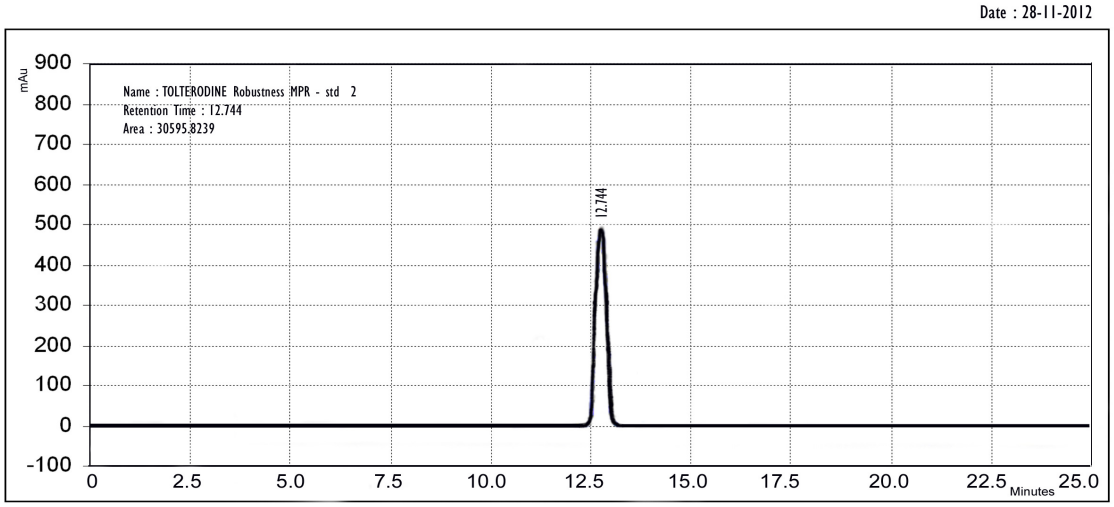


Fig 71: Robustness mobile phase(-) Standard 3(72:28)

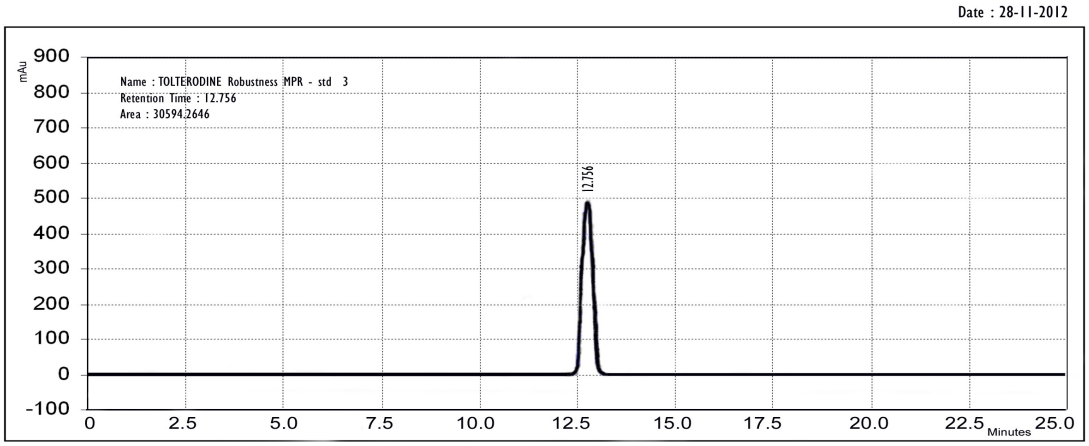


Fig 72: Robustness mobile phase(-) Sample 1(72:28)

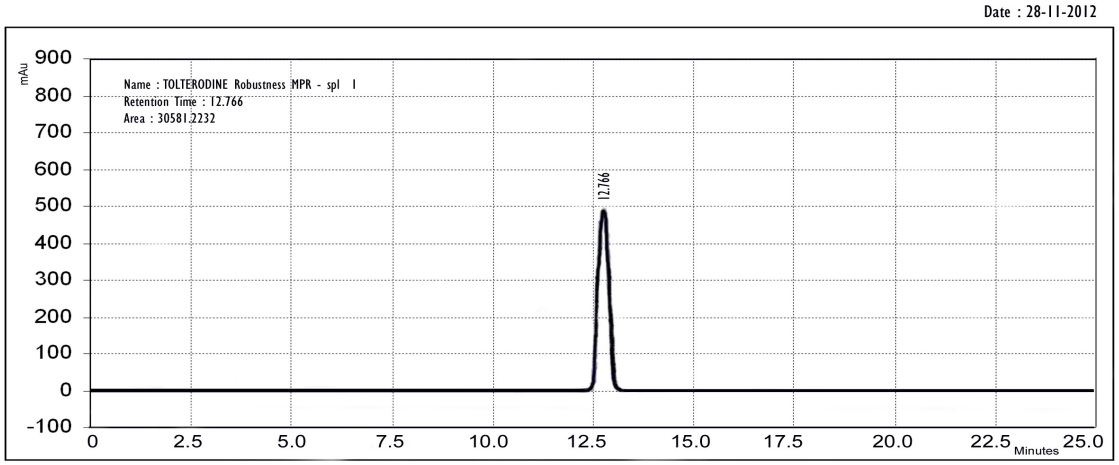


Fig 73: Robustness mobile phase(-) Sample 2(72:28)

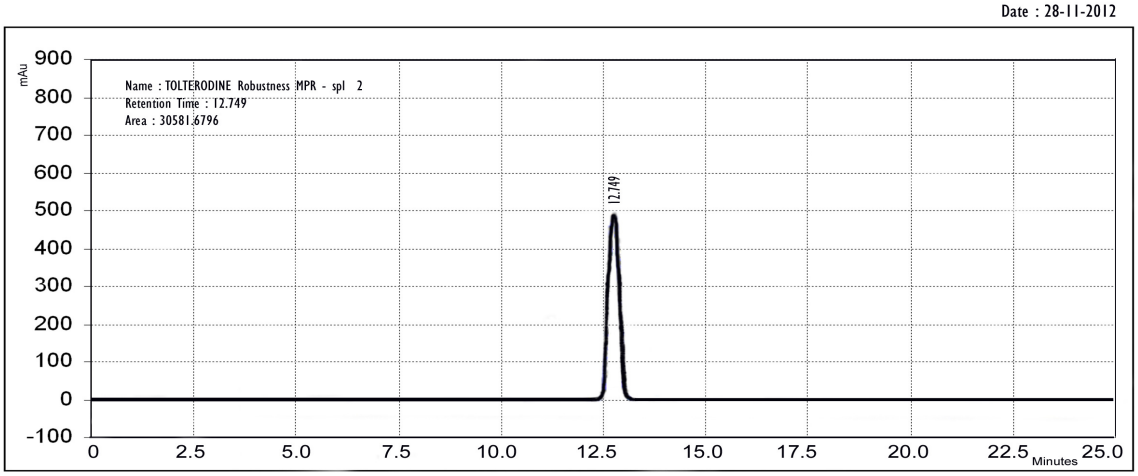


Fig 74: Robustness mobile phase(-) Sample 3(72:28)

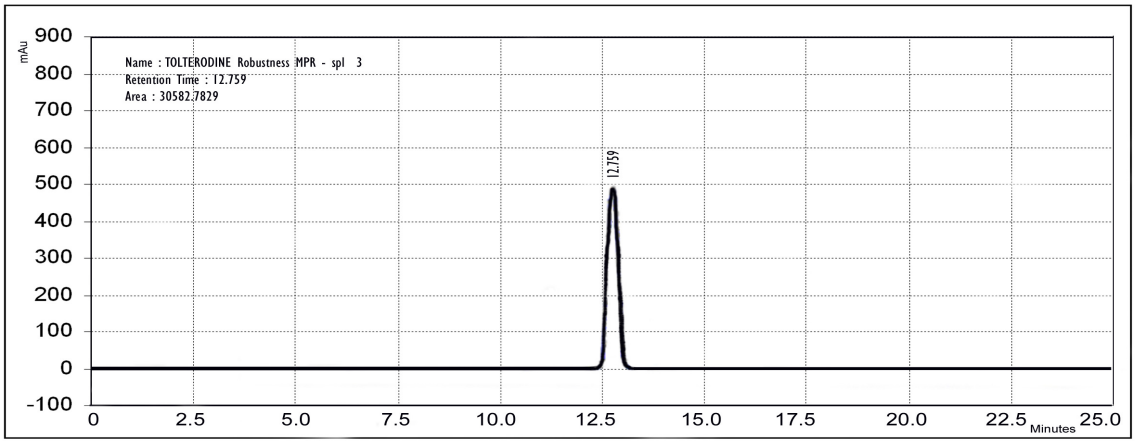


Fig 75: Robustness Flow Rate(+) Standard 1(1.1ml/min)

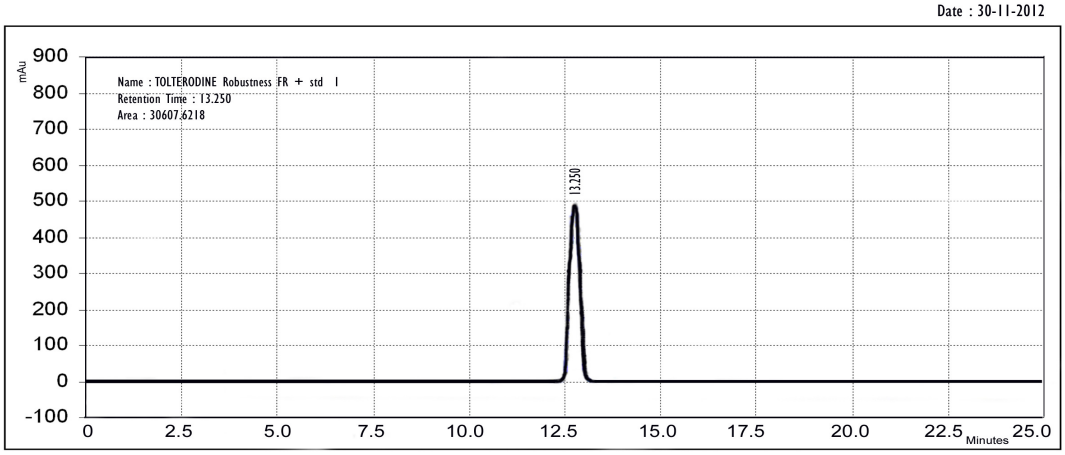


Fig 76: Robustness Flow Rate(+) Standard 2(1.1ml/min)

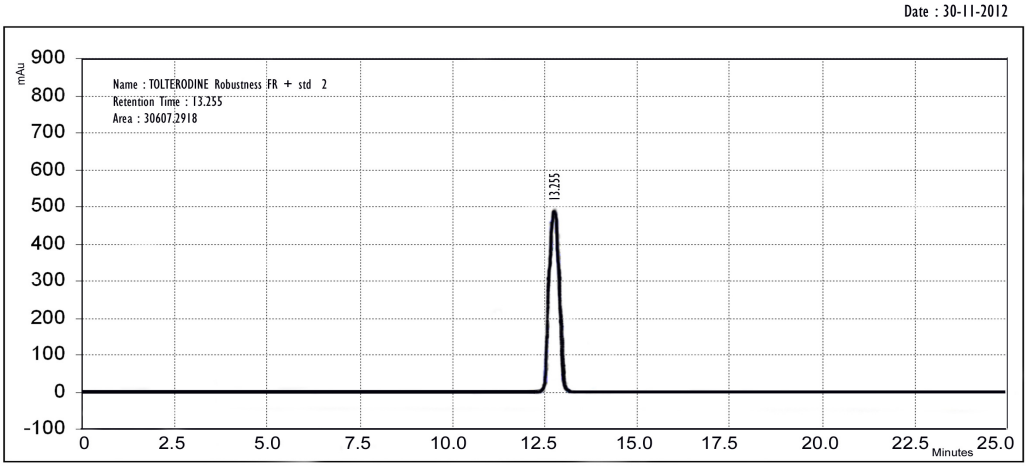


Fig 77: Robustness Flow Rate(+) Standard 3(1.1ml/min)

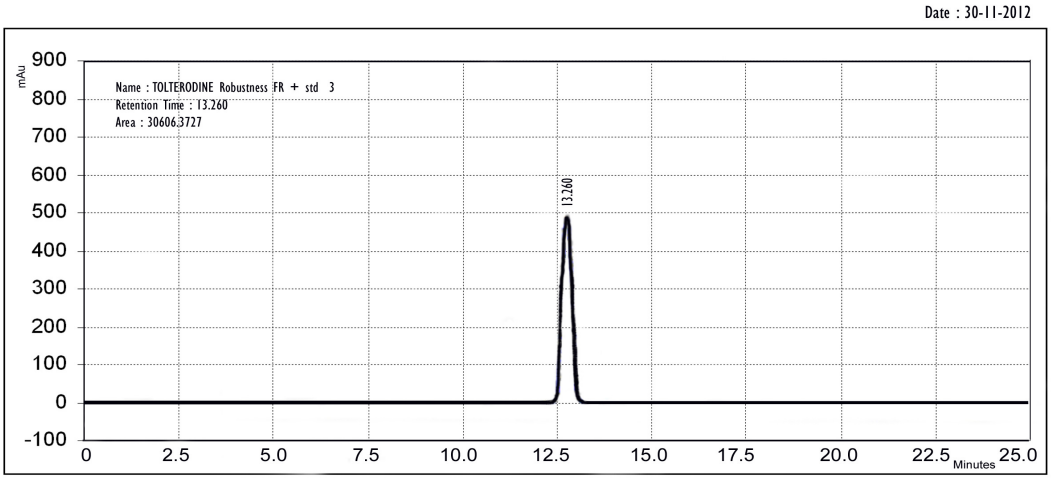


Fig 78: Robustness Flow Rate(+) Sample 1(1.1ml/min)

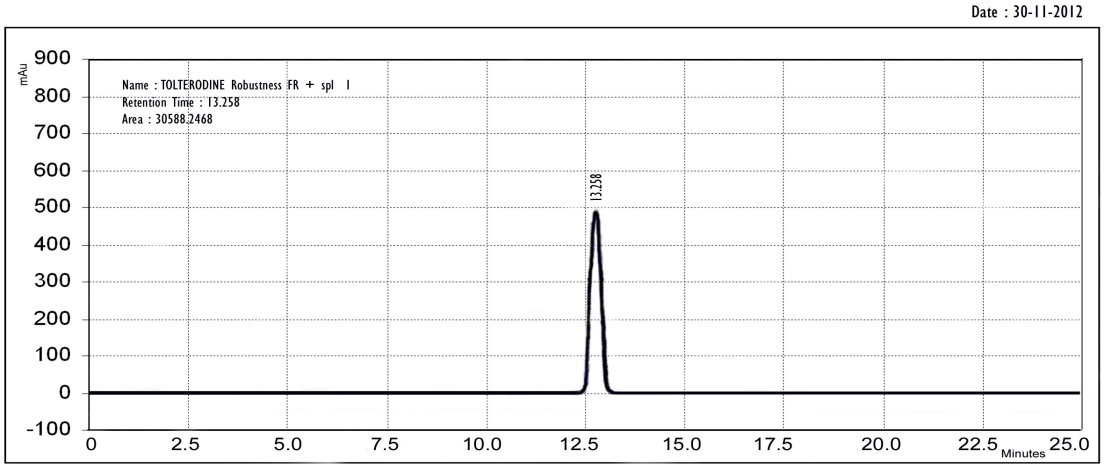


Fig 79: Robustness Flow Rate(+) Sample 2(1.1ml/min)

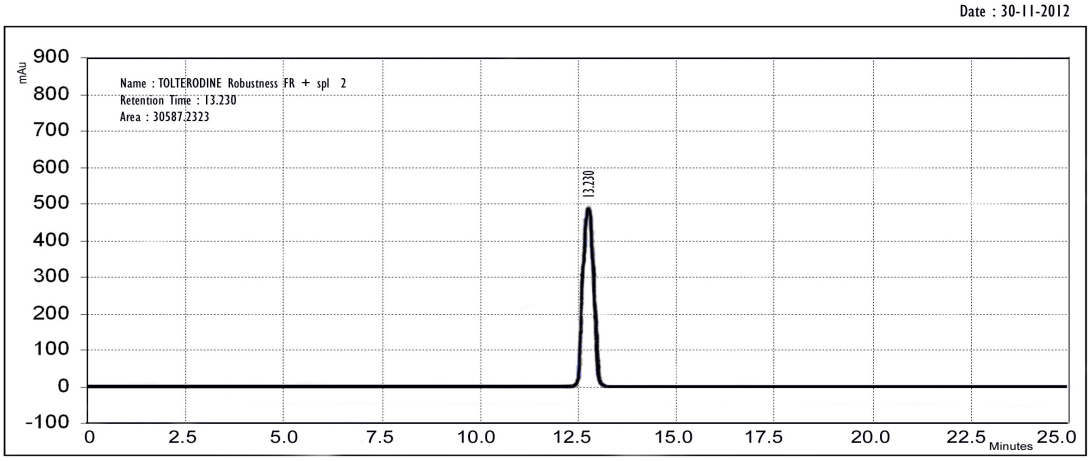


Fig 80: Robustness Flow Rate(+) Sample 3(1.1ml/min)

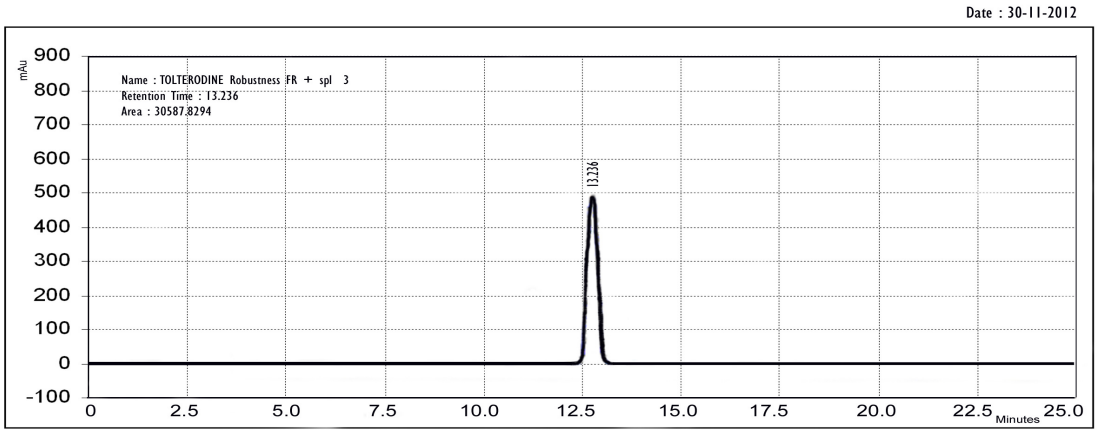


Fig 81: Robustness Flow Rate(-) Standard 1(0.9ml/min)

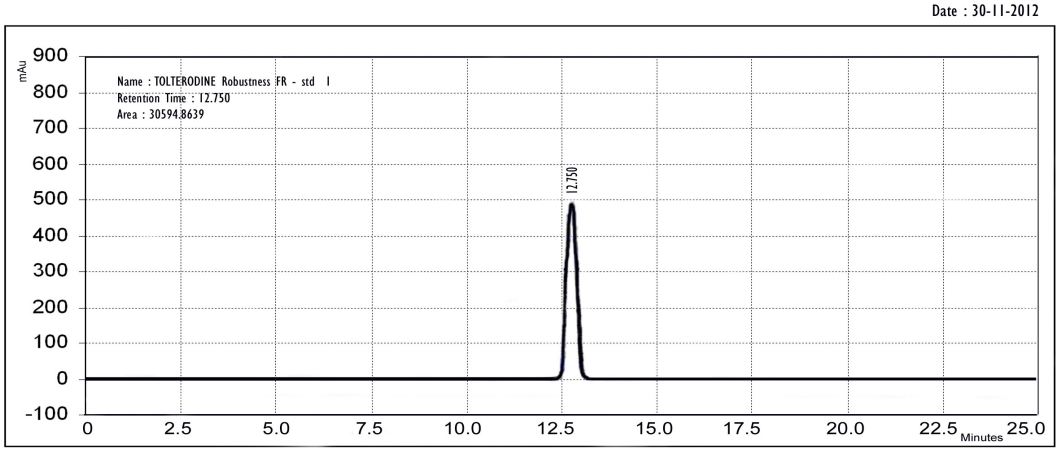


Fig 82: Robustness Flow Rate(-) Standard 2(0.9ml/min)

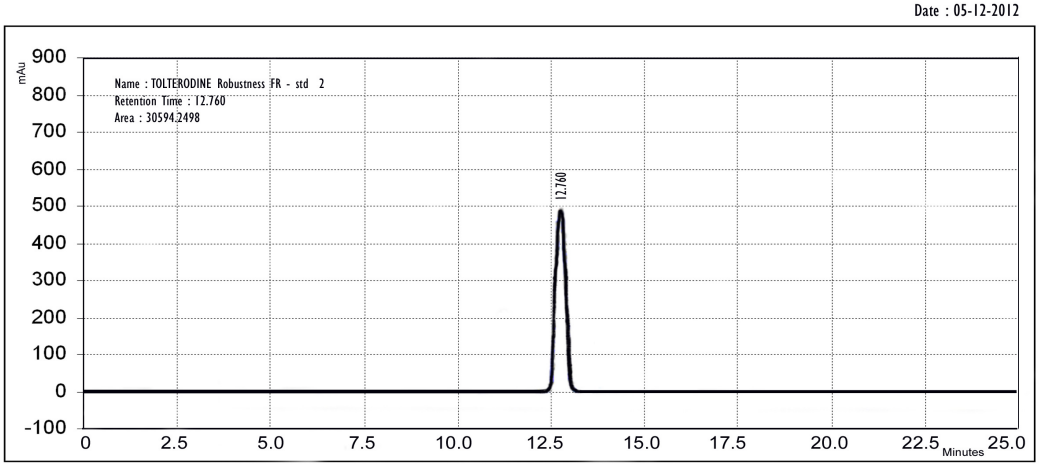


Fig 83: Robustness Flow Rate(-) Standard 3(0.9ml/min)

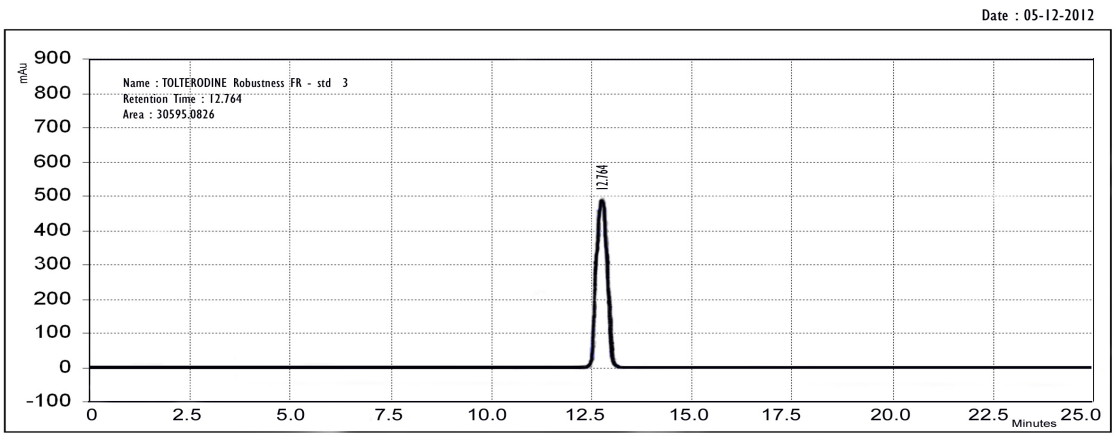


Fig 84: Robustness Flow Rate(-) Sample 1 (0.9ml/min)

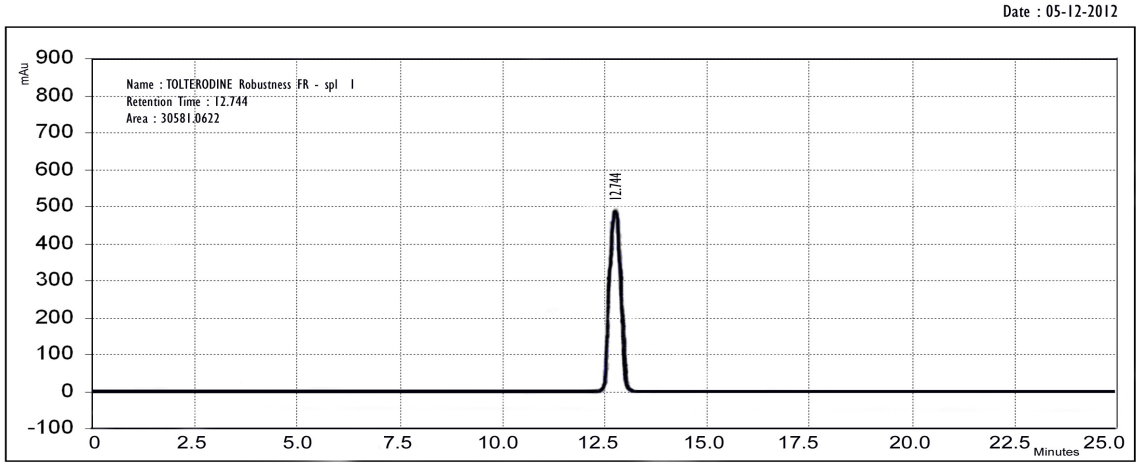


Fig 85: Robustness Flow Rate(-) Sample 2(0.9ml/min)

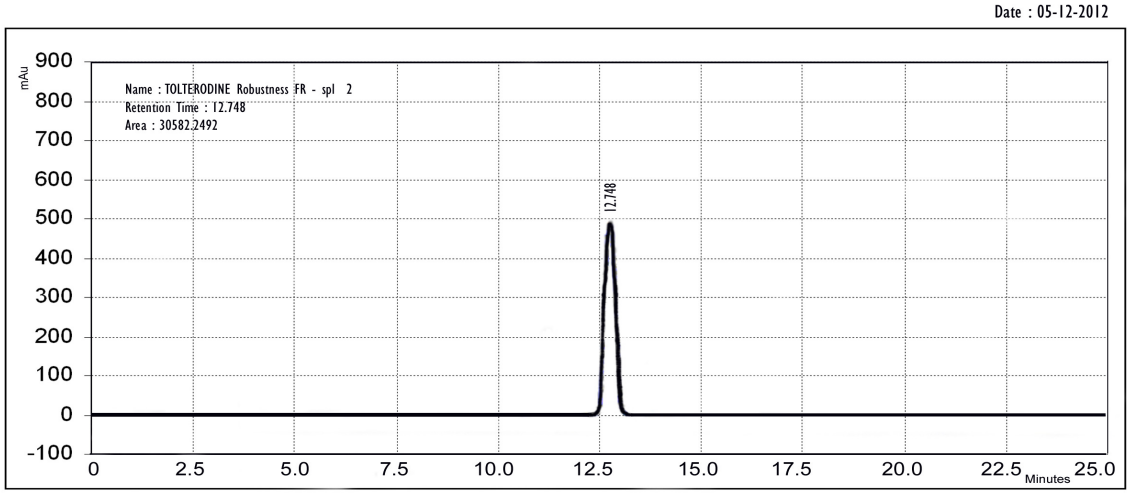


Fig 86: Robustness Flow Rate(-) Sample 3(0.9ml/min)

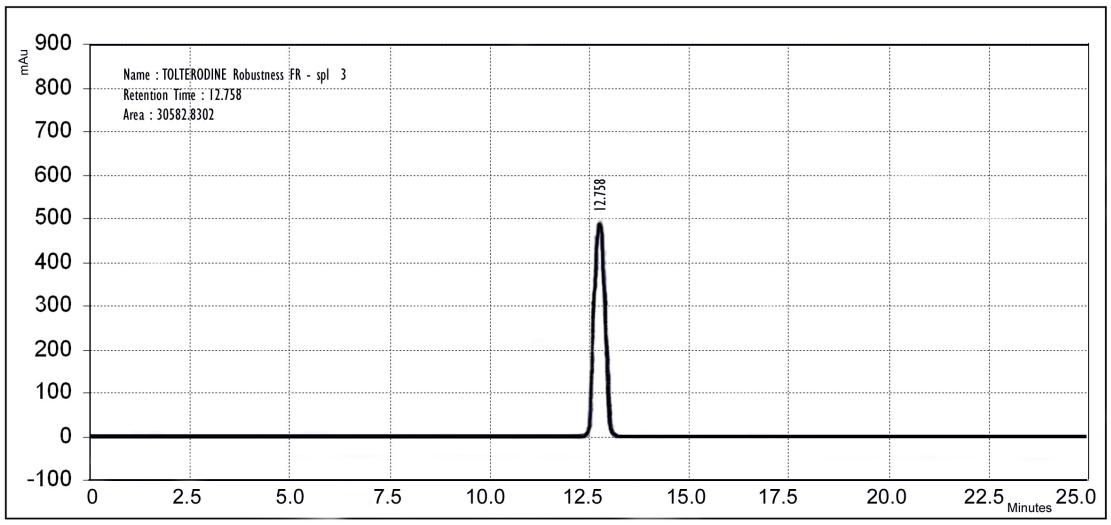


Fig 87: Blank

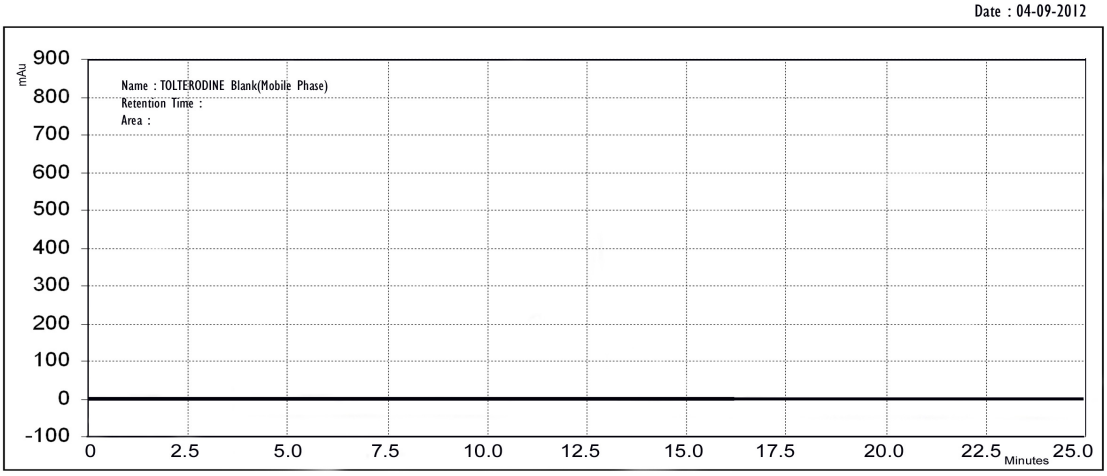


Fig 88: Placebo

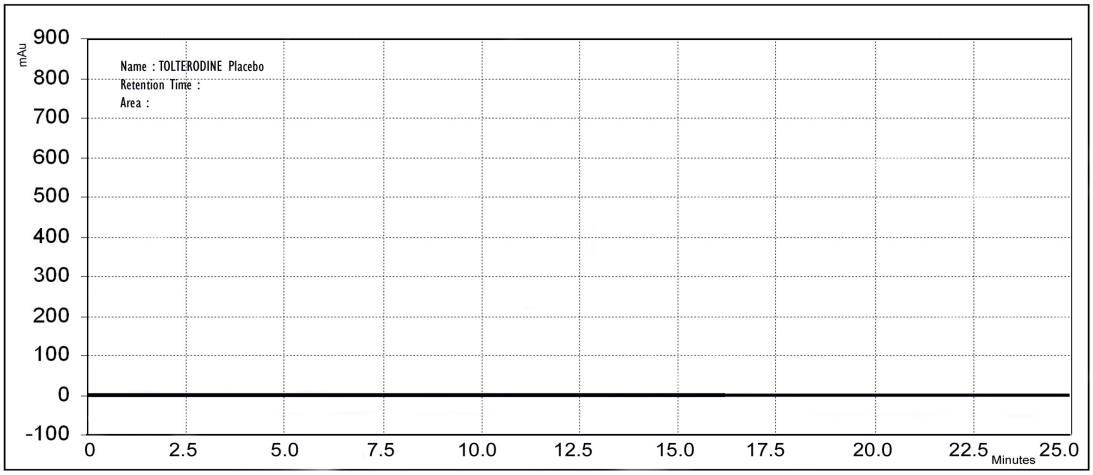


Fig 89: Standard 0 Hr

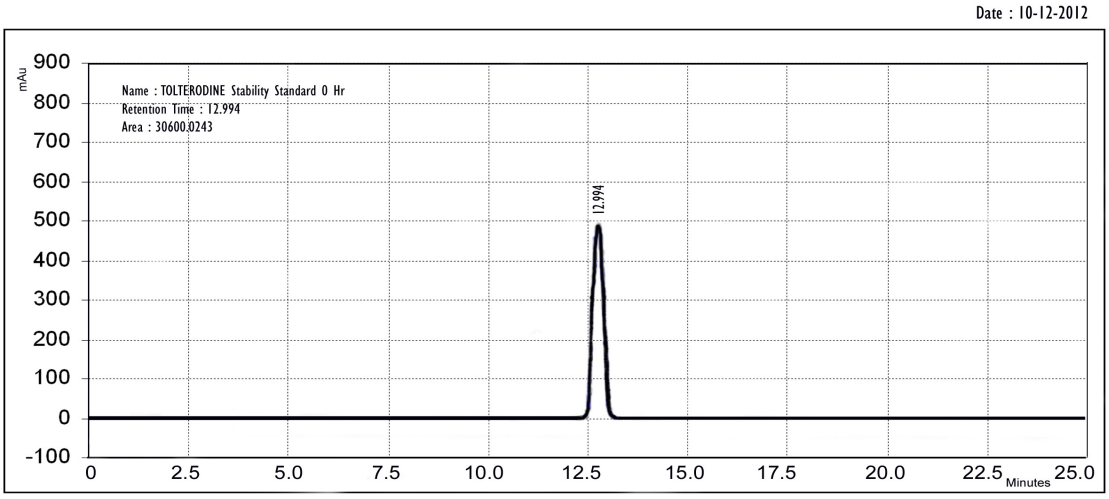


Fig 90: Sample 0 Hr

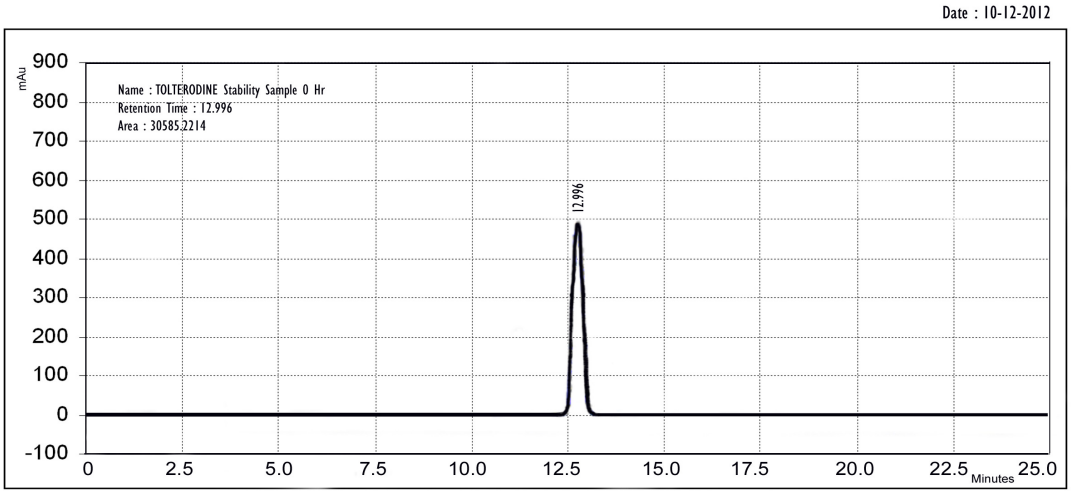


Fig 91: Standard 1 Hr

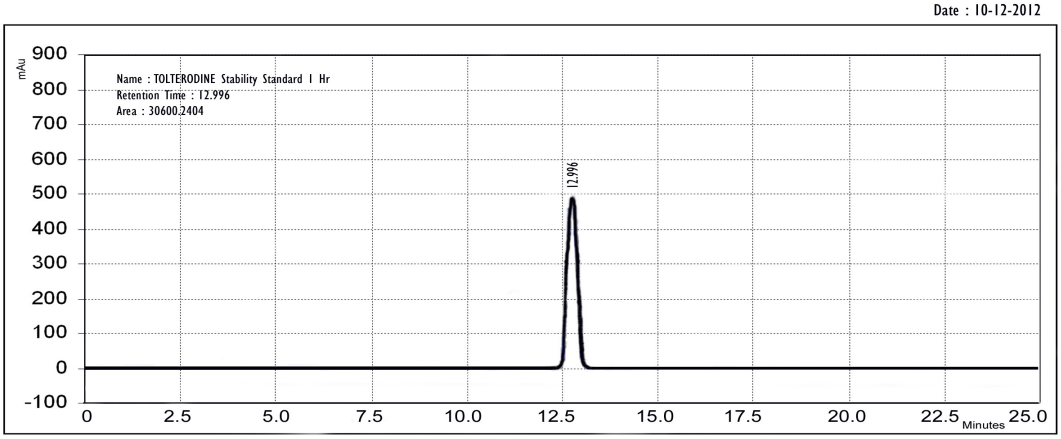


Fig 92: Sample 1 Hr

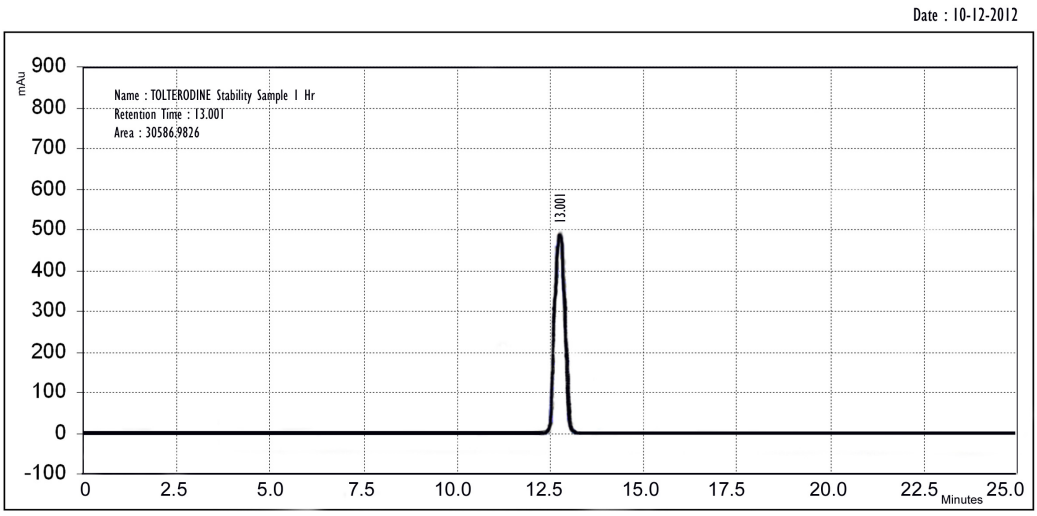


Fig 93: Standard 2 Hrs

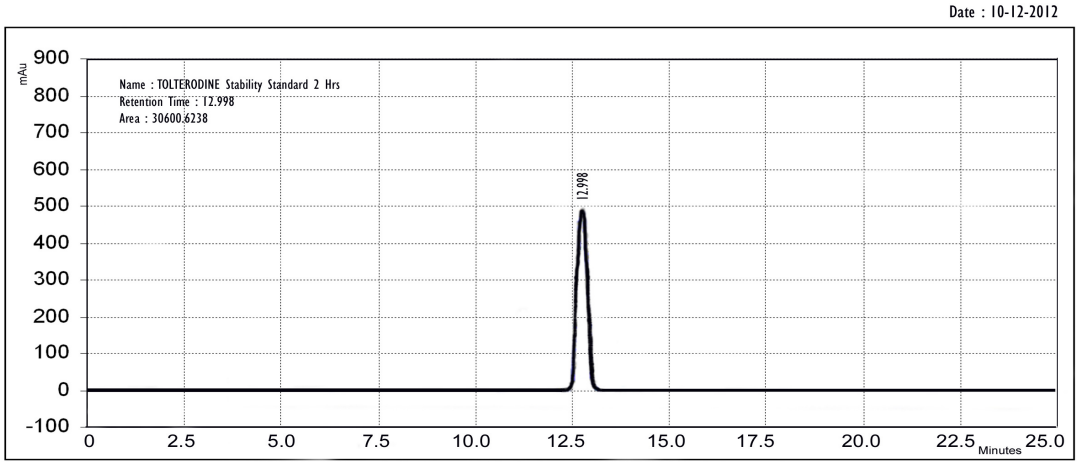


Fig 94: Sample 2 Hrs

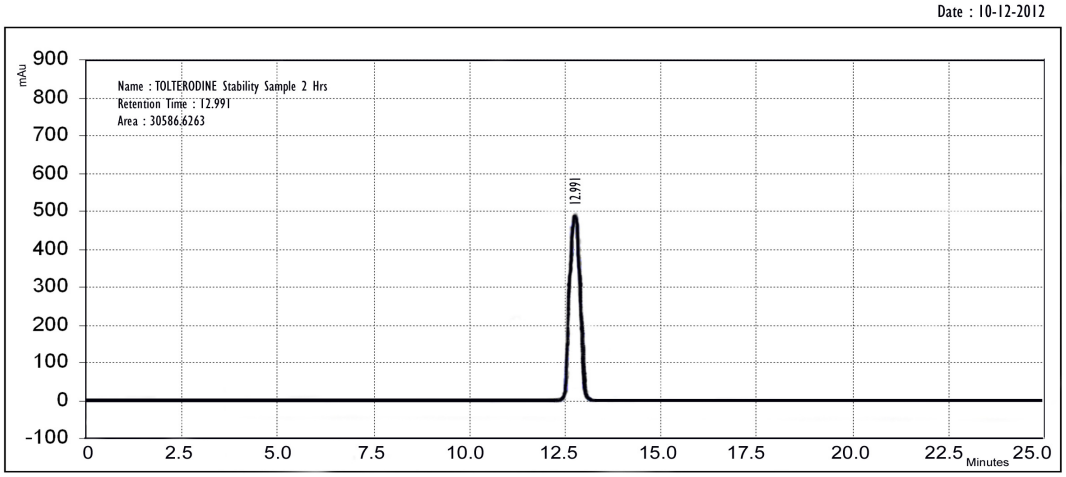


Fig 95: Standard 3 Hrs

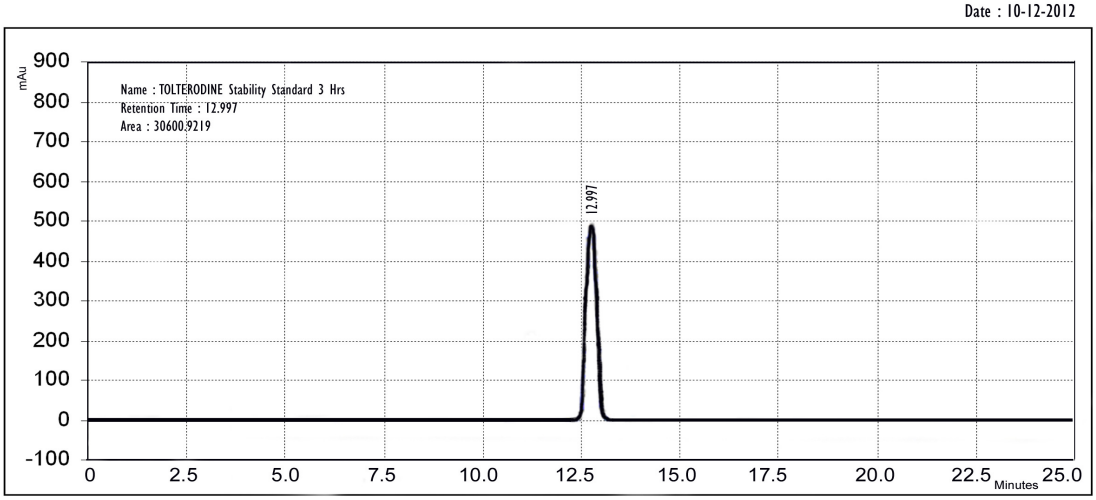


Fig 96: Sample 3 Hrs

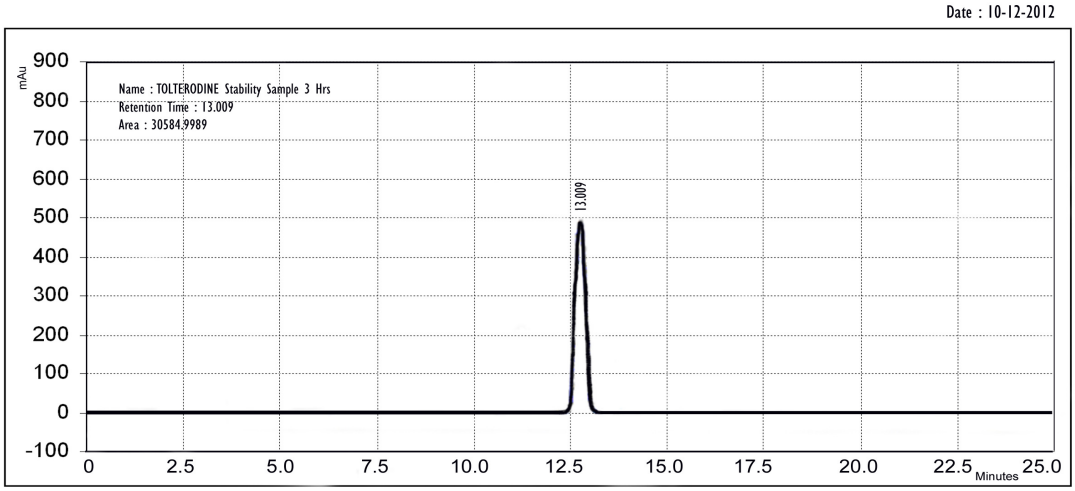


Fig 97: Standard 4 Hrs

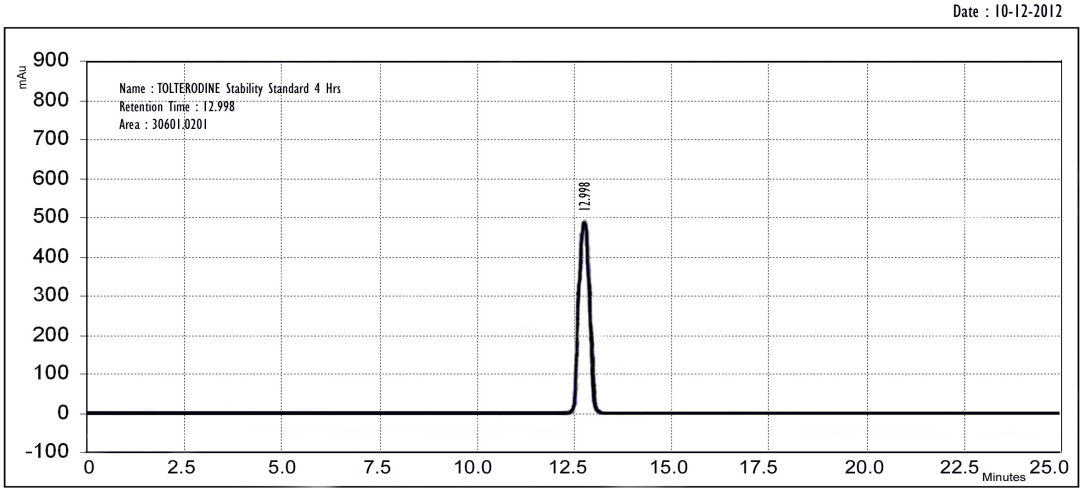


Fig 98: Sample 4 Hrs

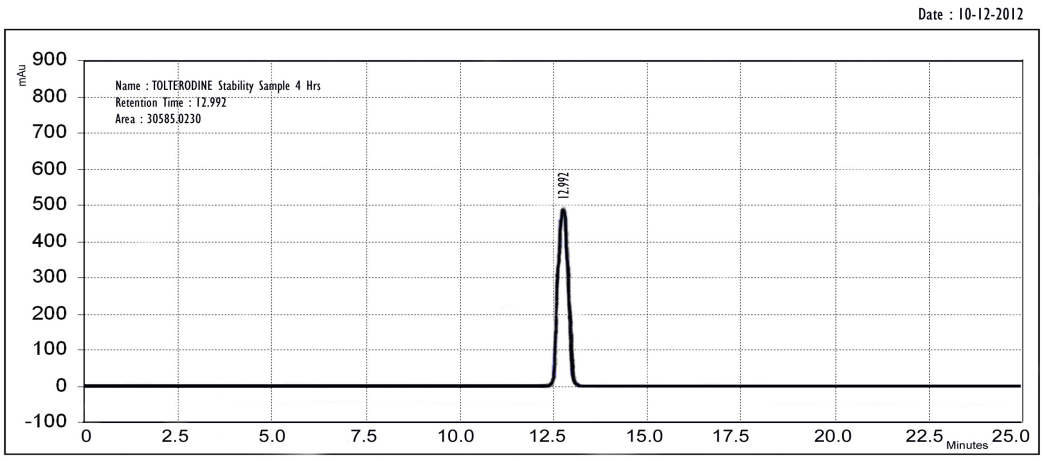


Fig 99: Standard 5 Hrs

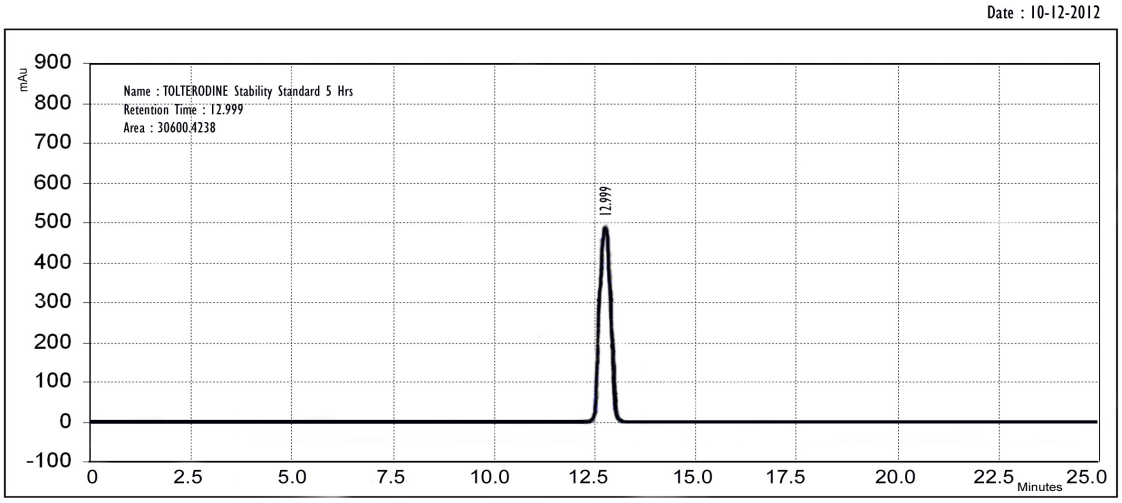


Fig 100: Sample 5 Hrs

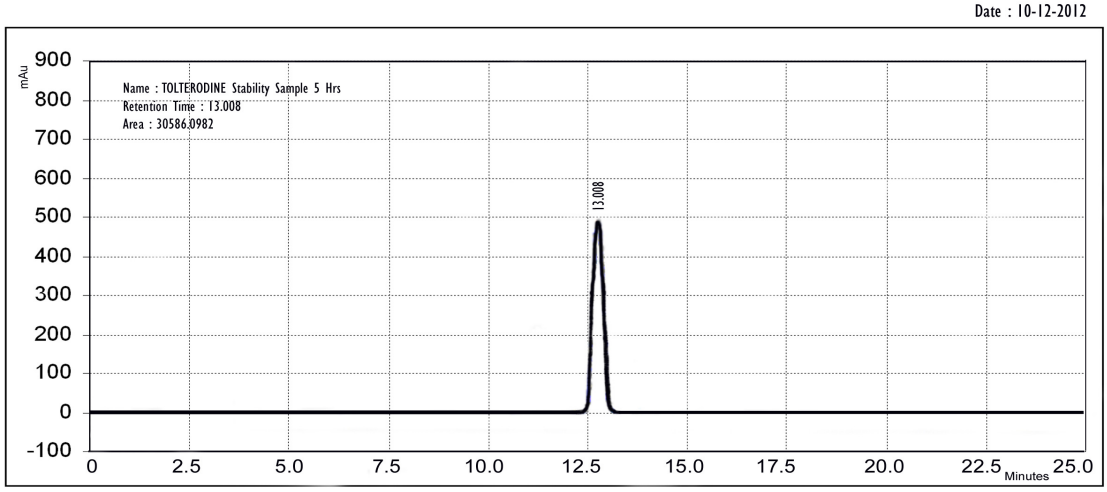


Fig 101: Standard 6 Hrs

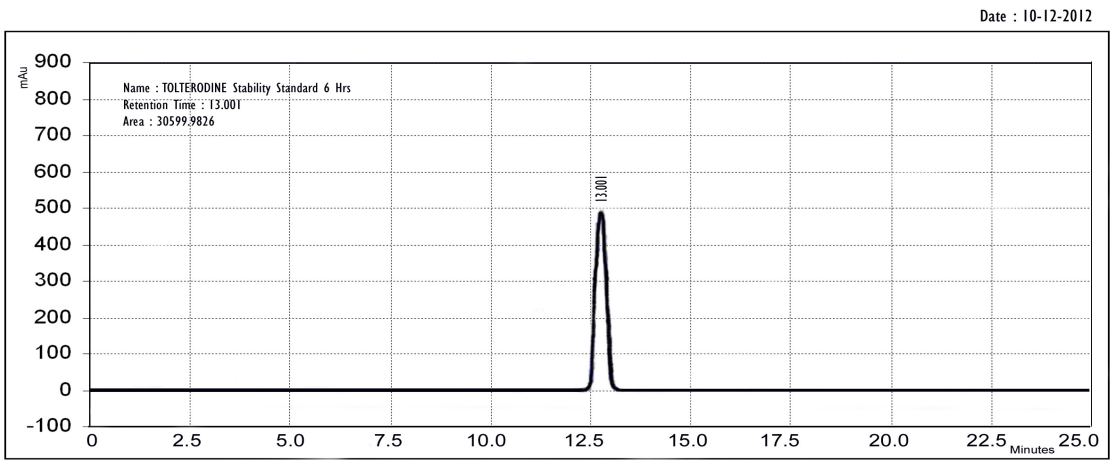


Fig 102: Sample 6 Hrs

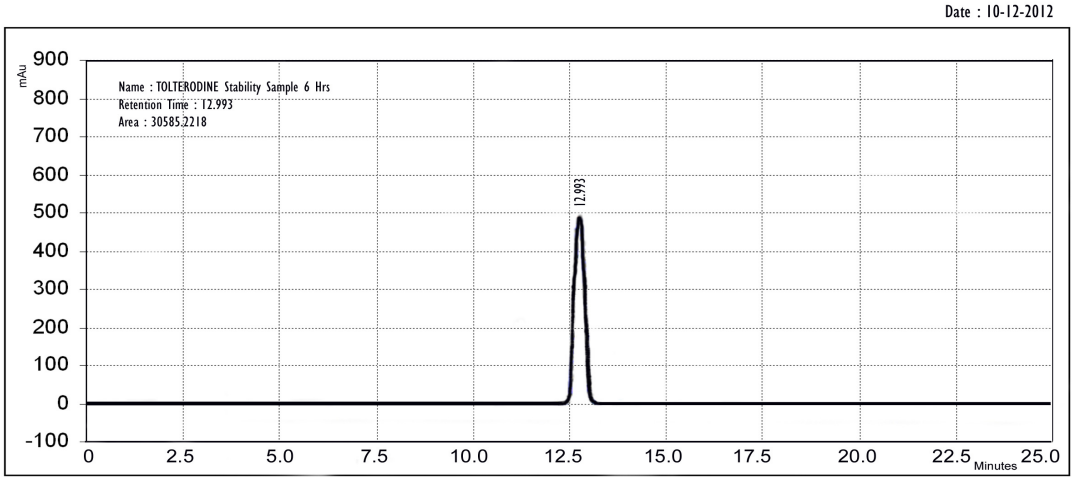


Fig 103: Standard 7 Hrs

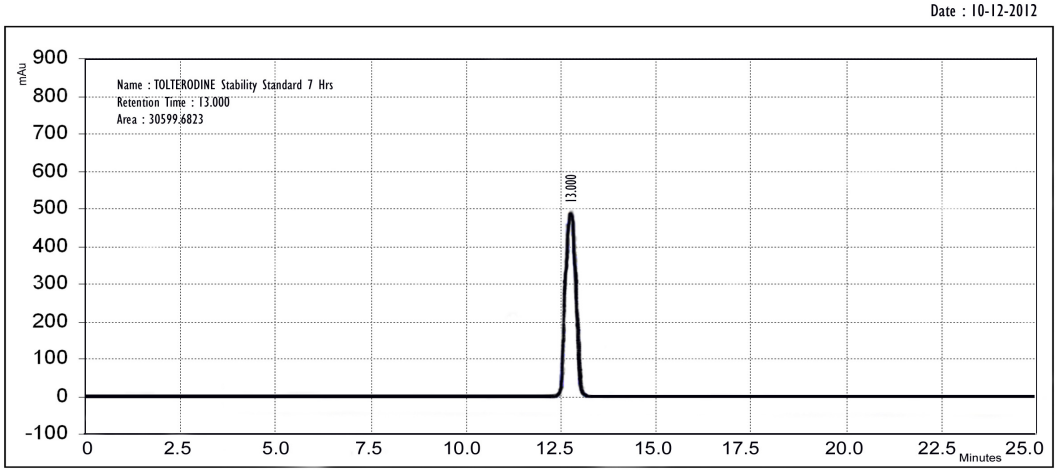


Fig 104: Sample 7 Hrs

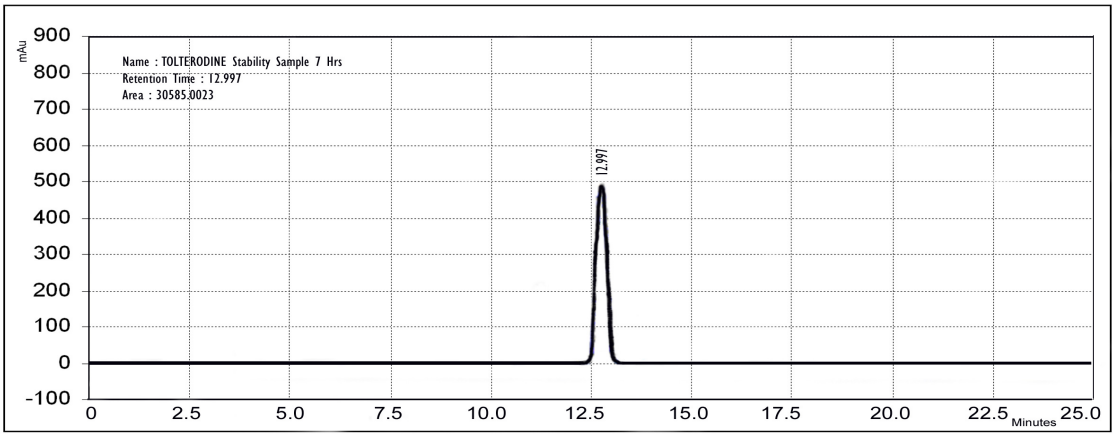


Fig 105: Standard 8 Hrs

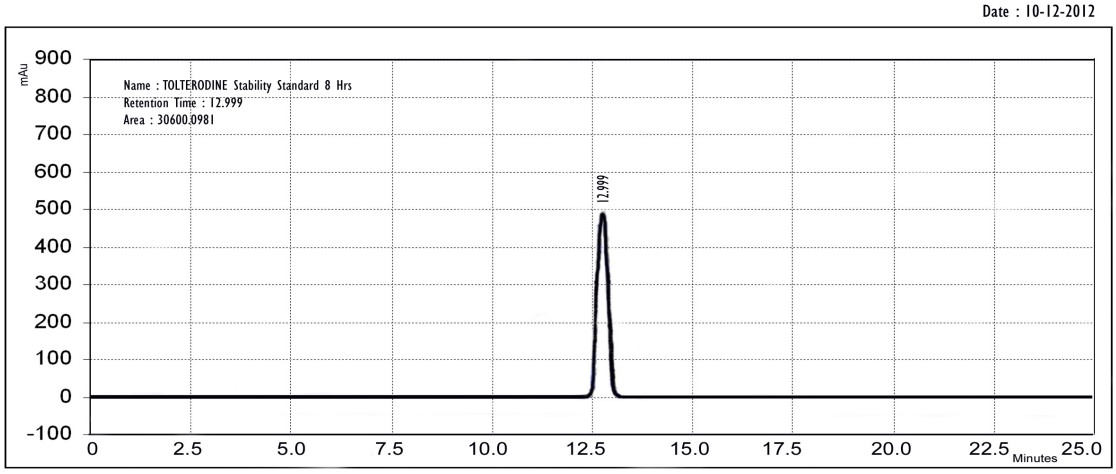


Fig 106: Sample 8 Hrs

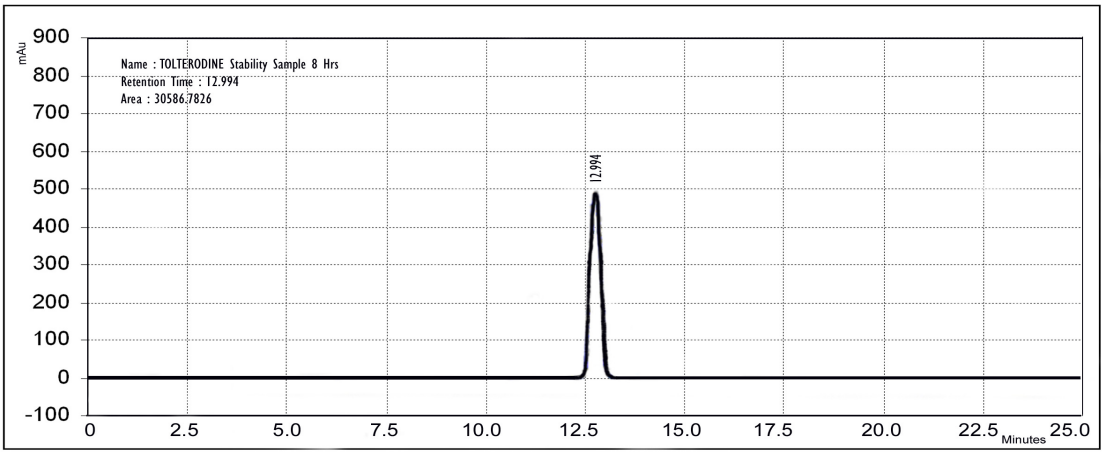


Fig 107: Standard 9 Hrs

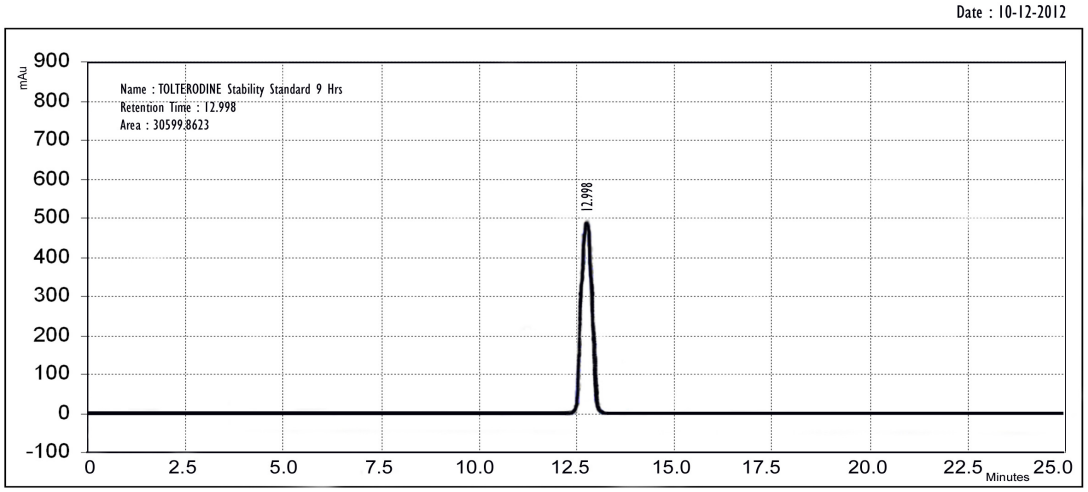


Fig 108: Sample 9 Hrs

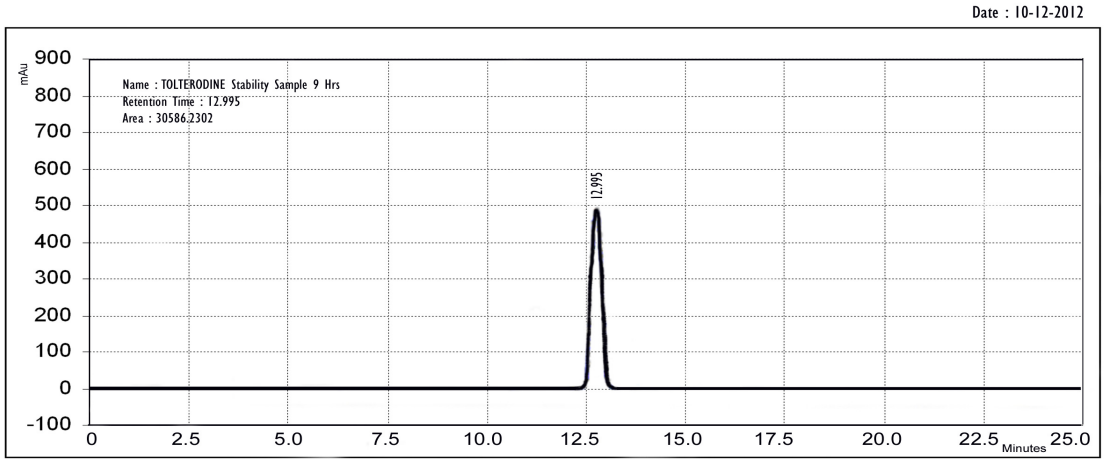
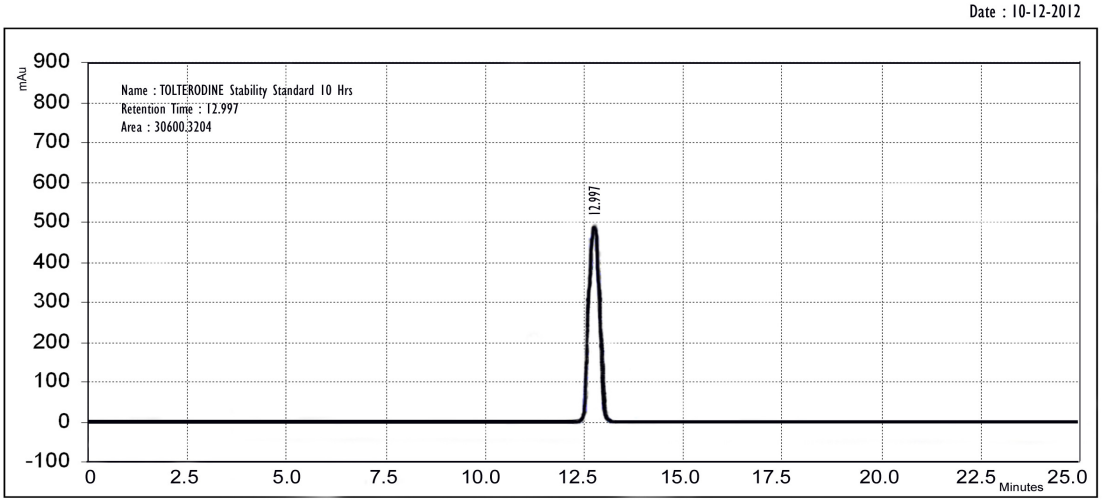


Fig 109: Standard 10 Hrs



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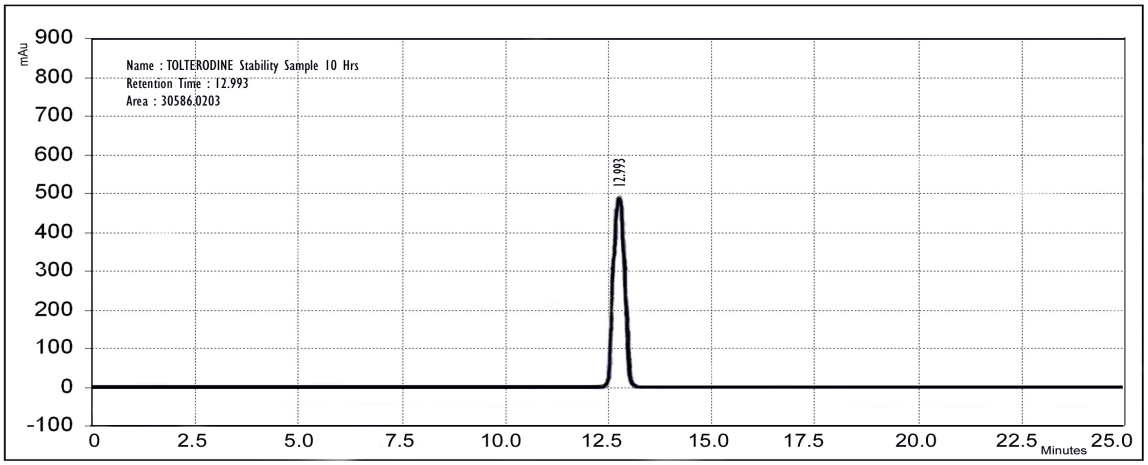


Fig 111: Standard 12 Hrs

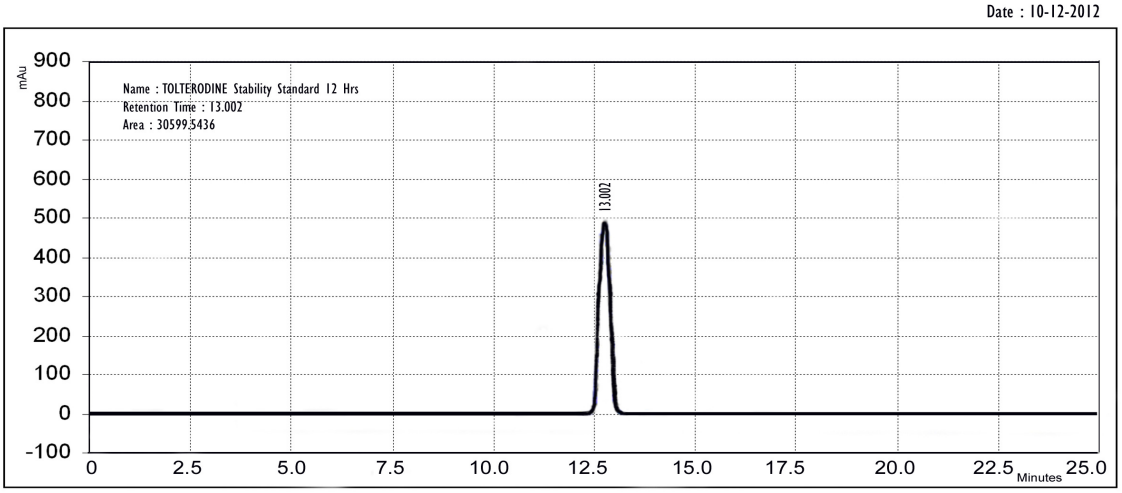


Fig 112: Sample 12 Hrs

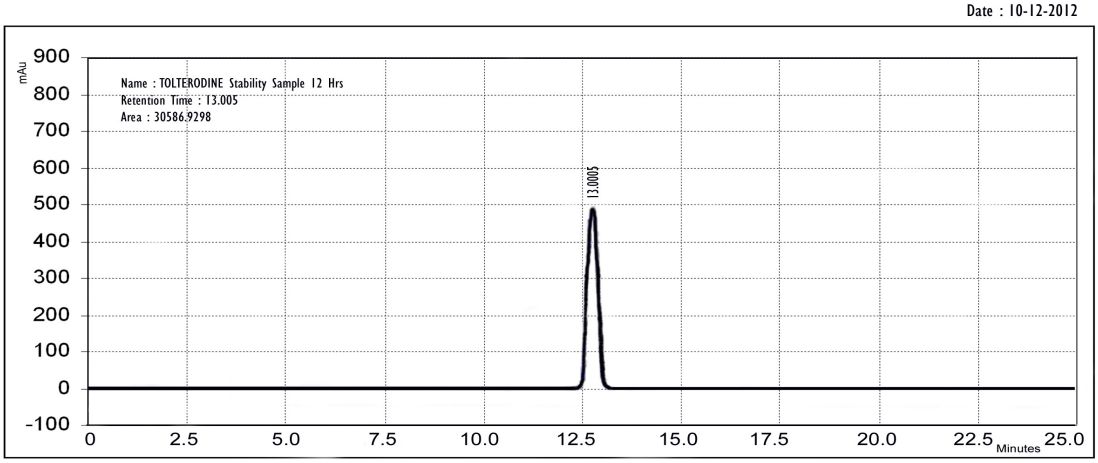


Fig 113: Standard 14 Hrs

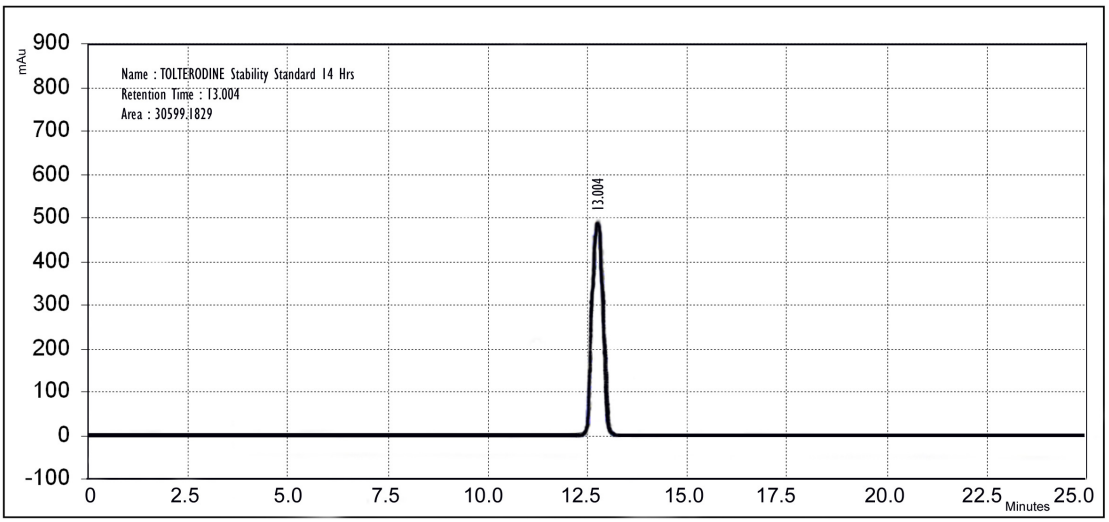


Fig 114: Sample 14 Hrs

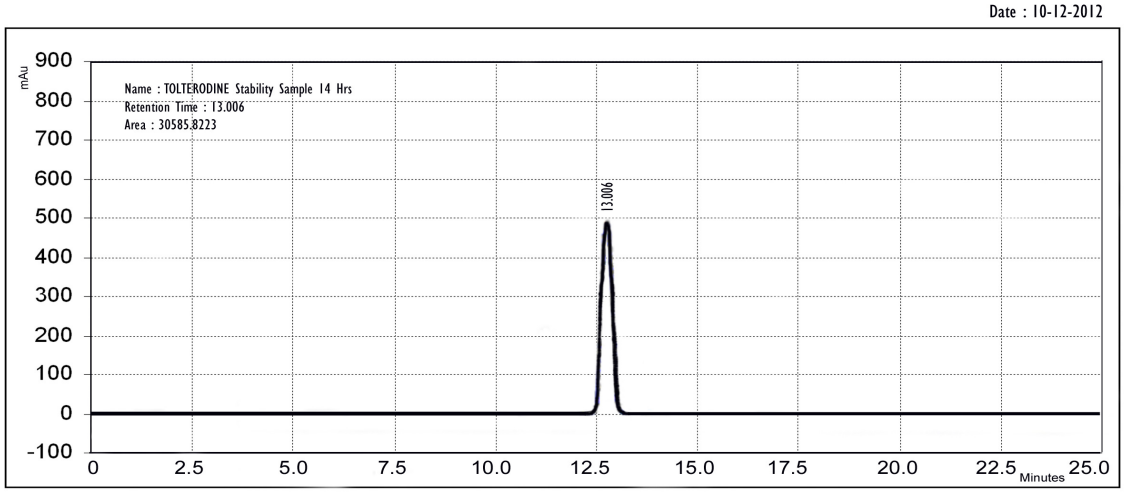


Fig 115: Standard 16 Hrs

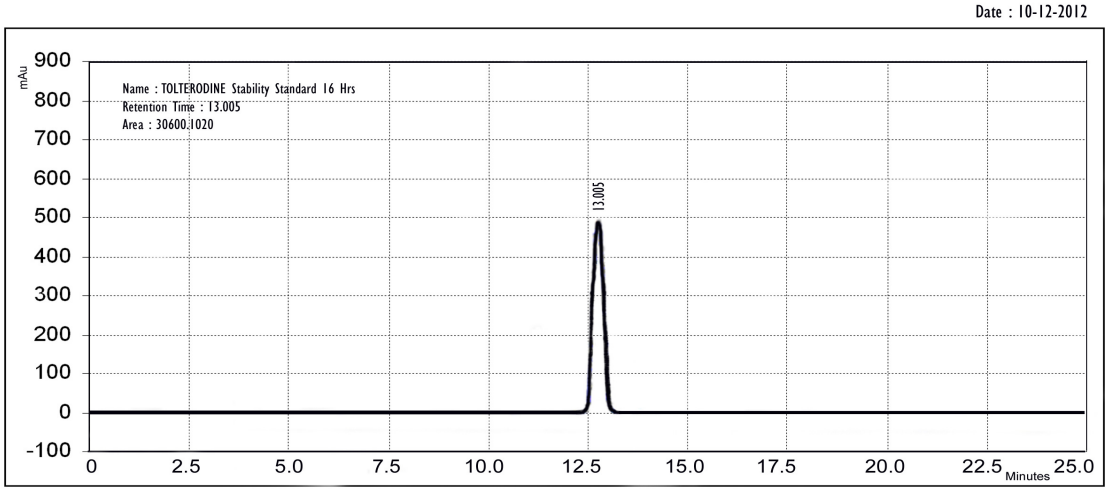


Fig 116: Sample 16 Hrs

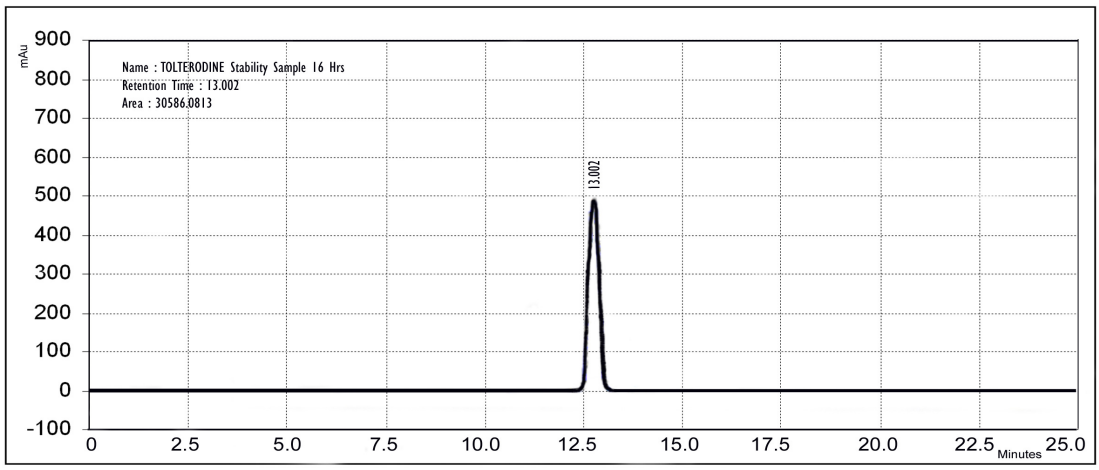


Fig 117: Standard 18 Hrs

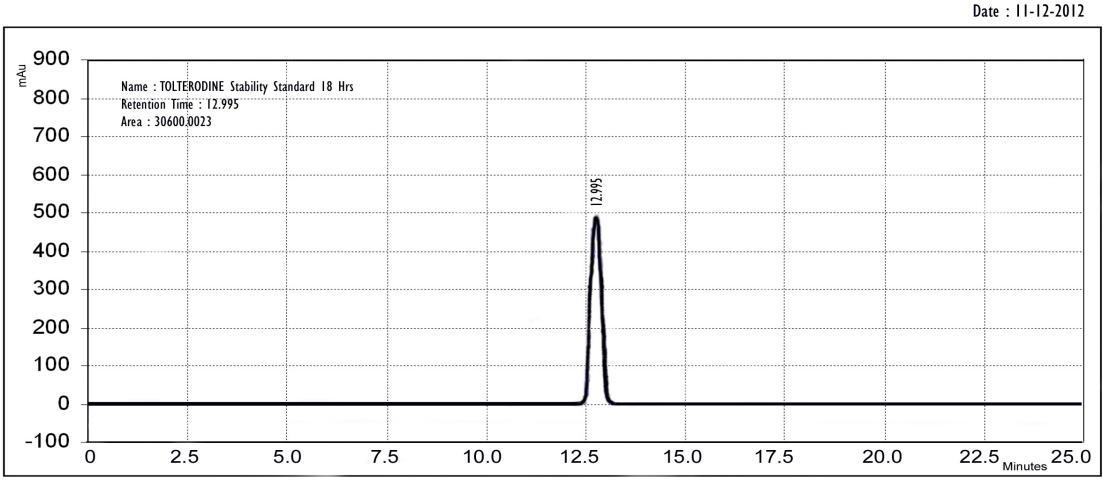


Fig118: Sample 18 Hrs

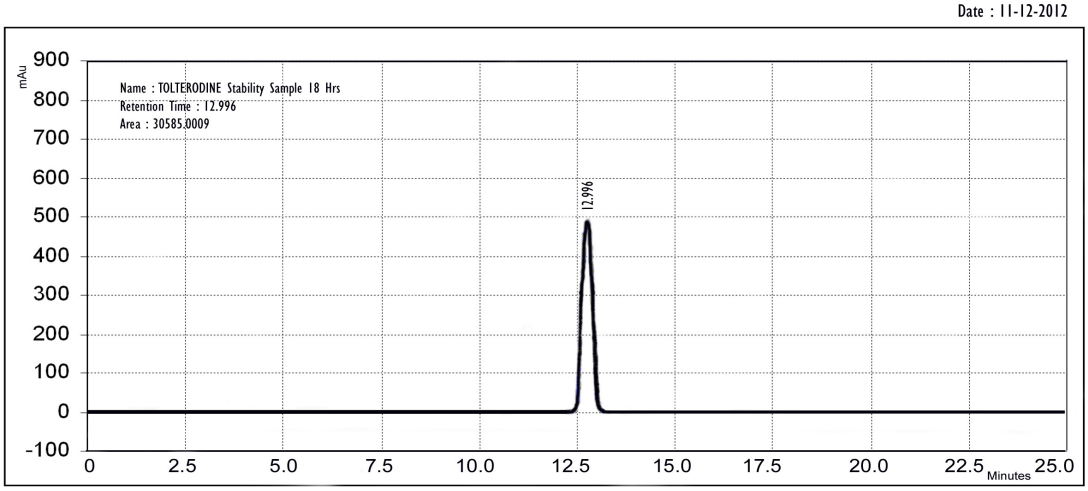


Fig 119: Standard 20 Hrs

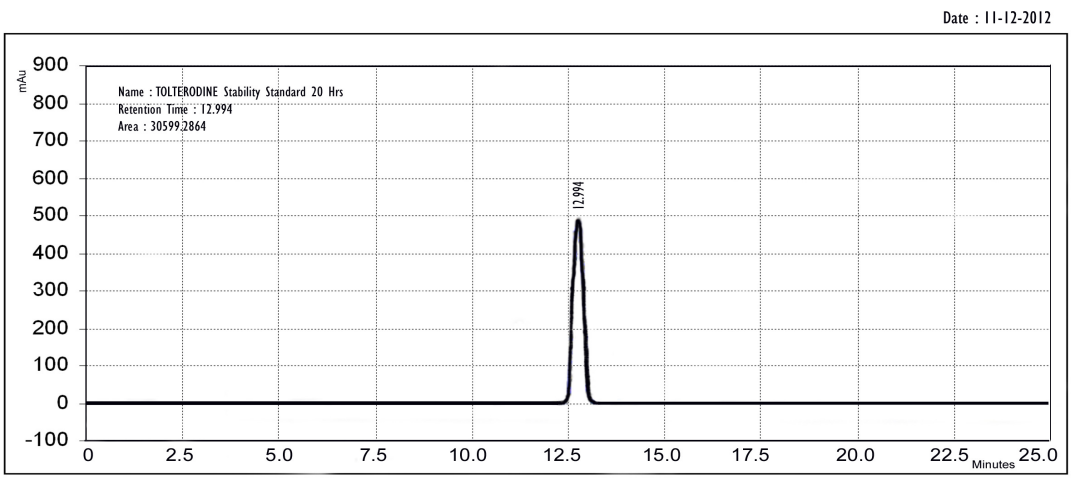


Fig 120: Sample 20 Hrs

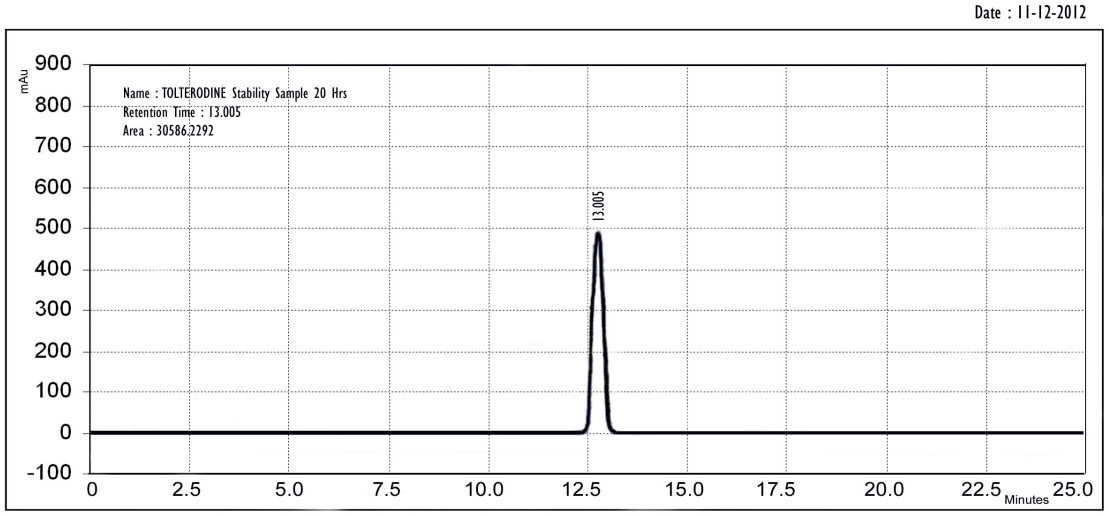


Fig 121 Standard 24 Hrs

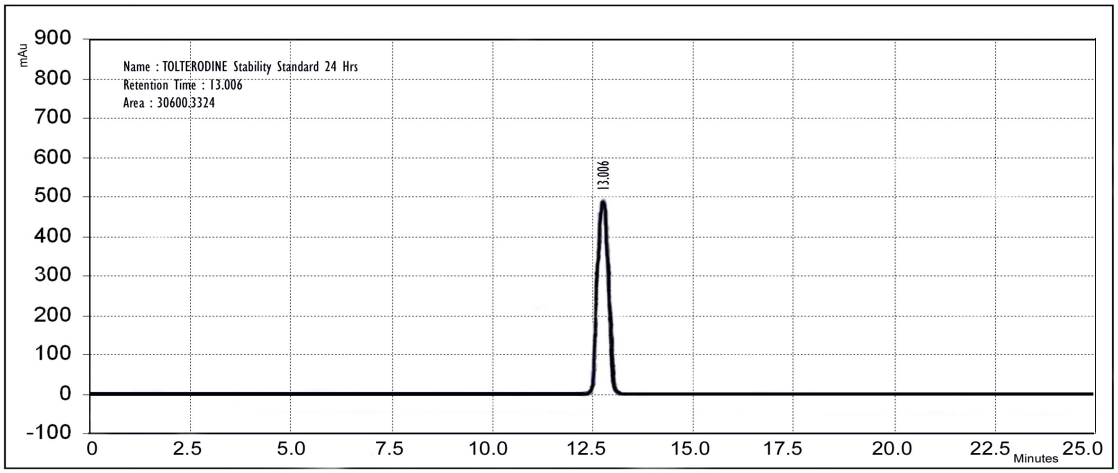


Fig 122: Sample 24 Hrs

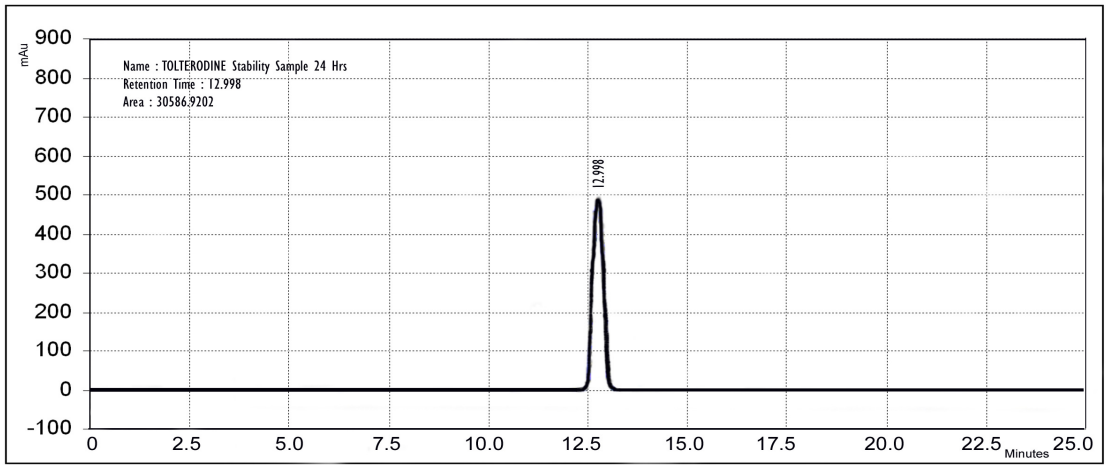


Fig 123: System suitability 1

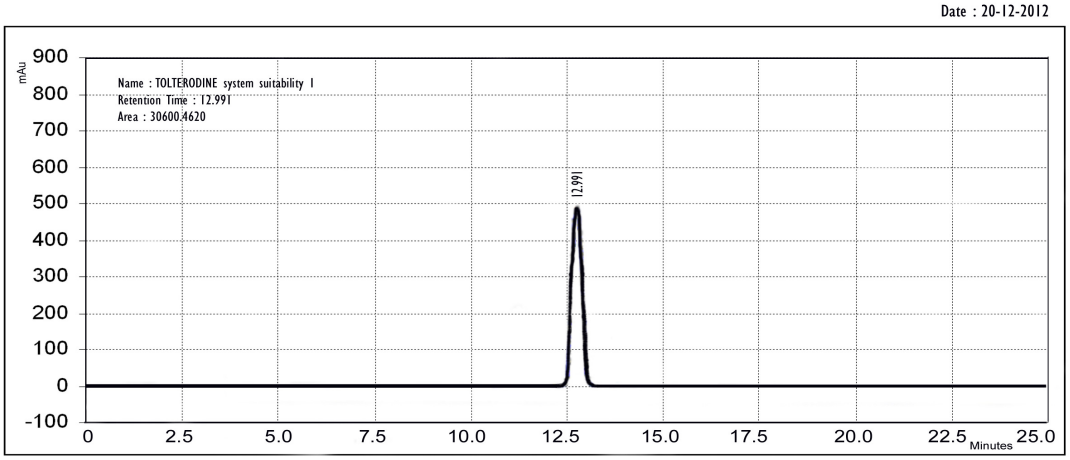


Fig 124: system suitability 2

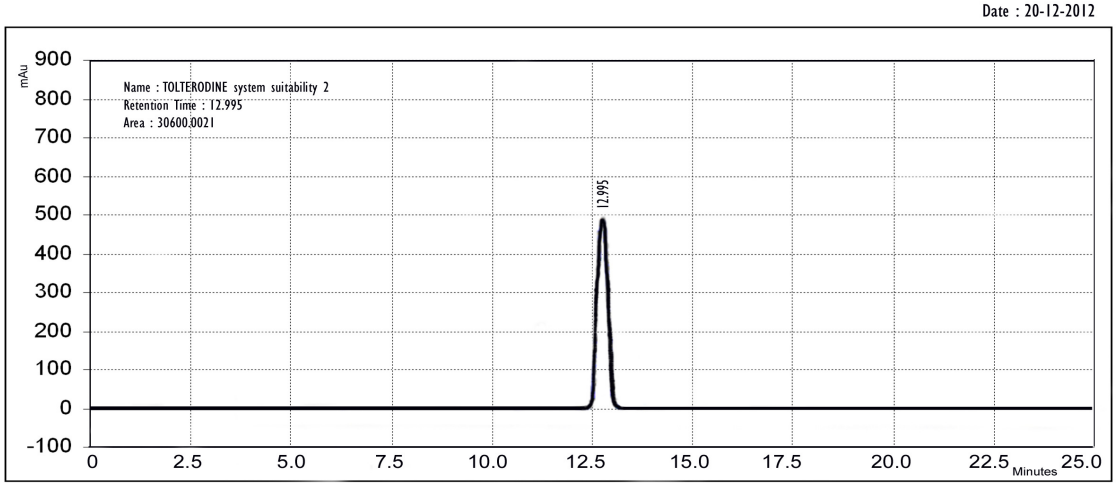


Fig 125: system suitability 3

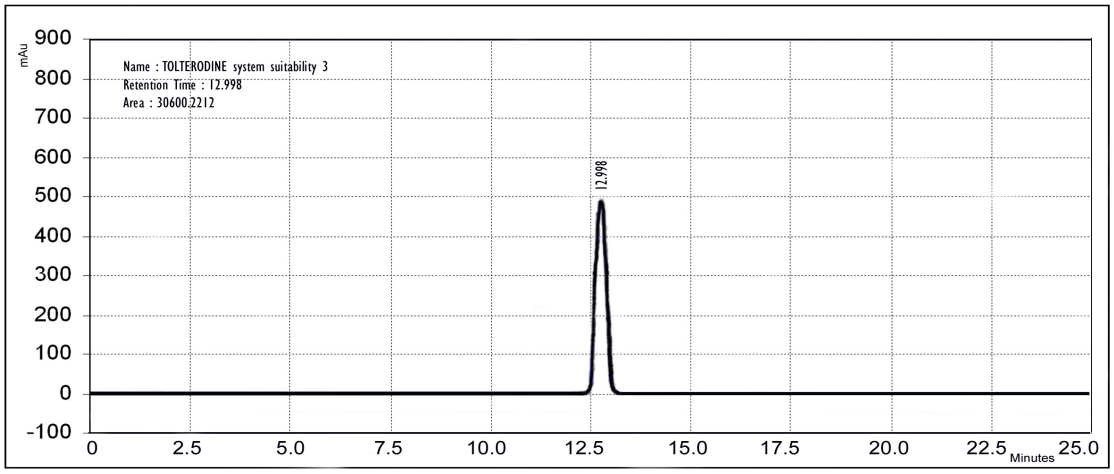


Fig 126:System suitability 4

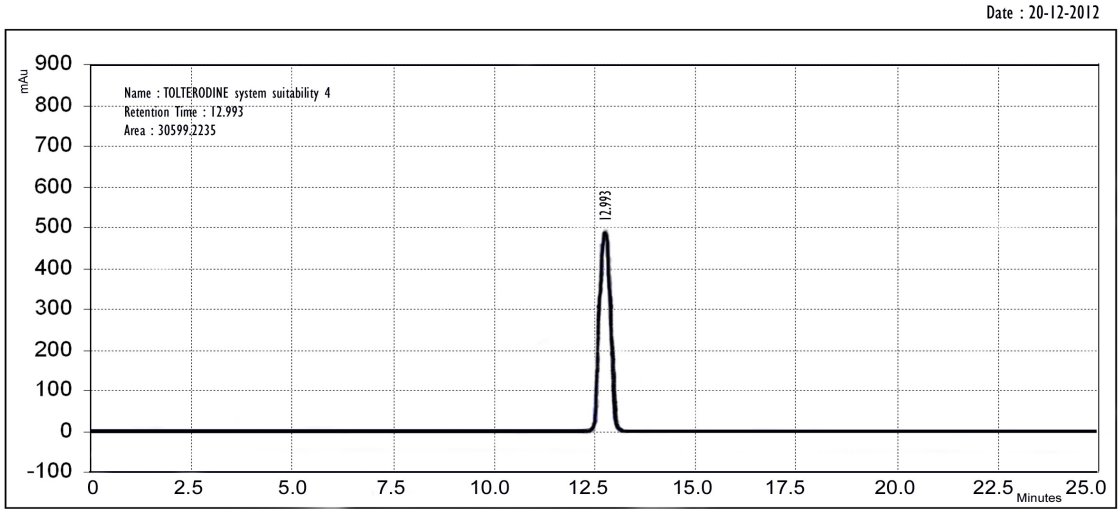


Fig 127:System suitability 5

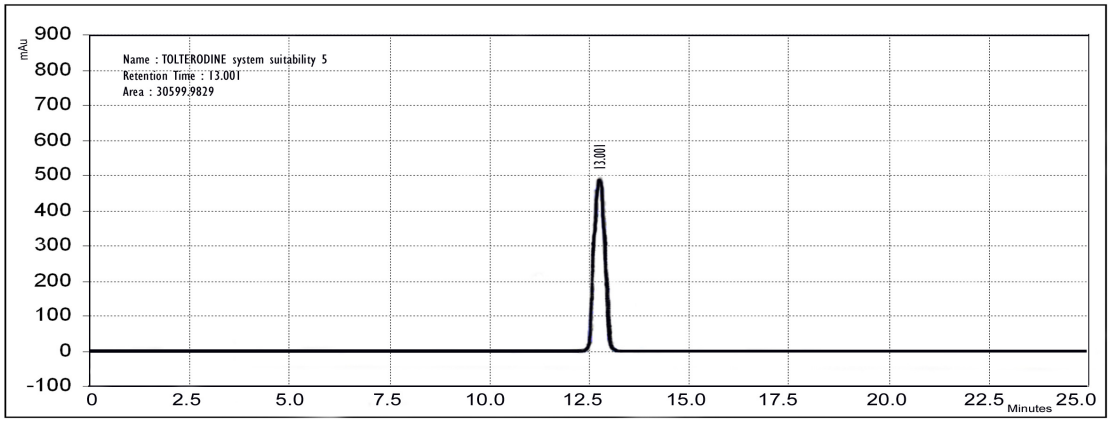
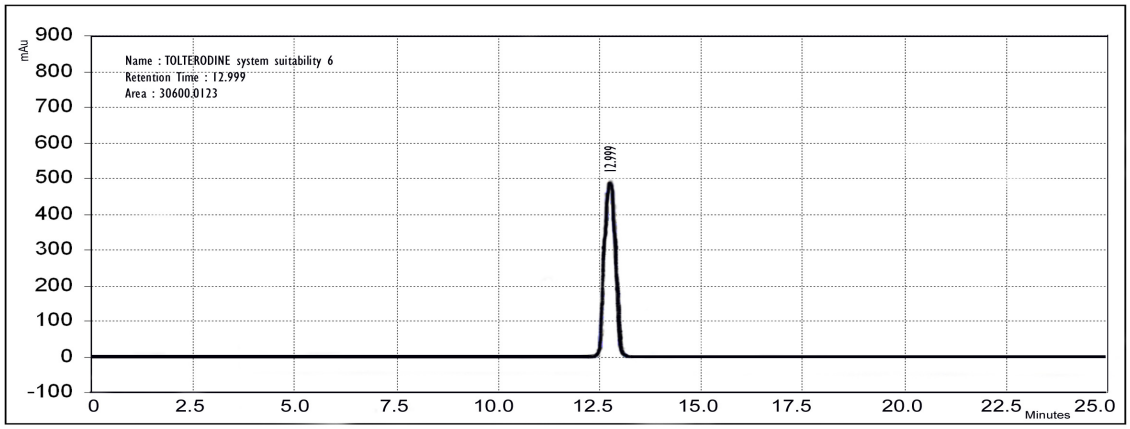


Fig 128:System suitability 6



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